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### EXTRACELLULAR VESICLES ISOLATED FROM STEM CELLS, AND USES THEREOF

#### Abstract

The present invention provides extracellular vesicles isolated from stem cells treated with zymosan, Poly I:C, and monophosphoryl lipid A (MPLA), their use for treatment of cancer, and a method for preparing the same. The extracellular vesicles according to the present invention contain various miRNAs and cytokines related to anticancer effects, and have tumor growth inhibitory effects. In addition, since the extracellular vesicles exhibit a synergistic effect in cancer treatment when administered in combination with an immune checkpoint inhibitor, a pharmaceutical composition for cancer treatment containing the extracellular vesicles as an active ingredient is highly likely to be effectively useful in cancer treatment by increasing immune activity in the body.

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## **Background/Summary**

### **TECHNICAL FIELD**

[0001] The present invention relates to novel extracellular vesicles isolated from stem cells treated with chemical substances, their use for treatment of cancer, and a method for preparing the same.

### **BACKGROUND ART**

[0002] Cancer is one of the major causes of death and morbidity worldwide, and various types of cancer treatment methods such as chemotherapy, surgery, and radiation therapy are being developed. However, these treatment methods have disadvantages such as causing serious side effects such as killing healthy cells or developing resistance. Another major disadvantage is that there is no effective therapeutic agent for solid cancer.

[0003] Recently, methods for treating cancer by activating the body's immune response have been reported. For example, various immune anticancer agents such as an immune checkpoint inhibitor, a chimeric antigen receptor-T cell (CAR-T), a T cell receptor modified T cell (TCR-T), a tumor infiltrating lymphocyte (TIL), a bispecific T-cell engager (BiTE), a tumor vaccine, and an oncolytic virus are being developed. However, unlike blood cancers, the effects of existing immune anticancer agents are insufficient in various solid cancers, because the tumor microenvironment surrounding cancer cells limits the function of immune cells.

[0004] Accordingly, methods for enhancing anticancer function by remodeling the tumor microenvironment are being developed, and these can be used alone or in combination with existing immune anticancer agents. In addition, these immune anticancer agents can be used in combination with various cancer treatment methods such as existing chemotherapy, surgery, and radiation therapy.

[0005] Meanwhile, extracellular vesicles are nanometer-sized vesicles composed of lipid bilayers, and comprise various types of physiologically active substances such as proteins, lipids, RNA (including microRNA, mRNA, and lncRNA) that exist inside the parent cell, and are known to play a very important role in the transmission of information between cells. In particular, extracellular vesicles isolated from stem cells are attracting attention as an alternative that can overcome various shortcomings of cell therapeutic agents because they have the therapeutic efficacy of stem cells while being cell-free.

[0006] Extracellular vesicles are released or secreted from various cells of animals, plants, fungi, algae, etc., and are dispersed, suspended, precipitated, floating, or mixed in biological solutions such as cell culture solution, cell culture supernatant, stem cell culture solution, stem cell culture supernatant, whole blood, serum, umbilical cord blood, plasma, ascites fluid, brain and cerebrospinal fluid, placental extract, and bone marrow aspirate. As such, extracellular vesicles present in biological solutions can be separated and purified by various separation methods.

[0007] Currently known methods for isolating extracellular vesicles include ultracentrifugation, density gradient centrifugation, ultrafiltration, tangential flow filtration, size exclusion chromatography, ion exchange chromatography, immunoaffinity capture, microfluidics-based isolation, exosome precipitation, total exosome isolation kit, or polymer-based precipitation.

[0008] In addition, it is known that the characteristics and functions of extracellular vesicles differ

depending on the origin and type of the cell, the state of cell growth, proliferation, differentiation, death, immortalization, etc., and the characteristics and functions may also vary depending on the culture state of the cell, culture conditions, and the isolation method and conditions of the extracellular vesicles, etc. Therefore, specific culture conditions can be used to prepare extracellular vesicles with specific physiological activities.

[0009] The incorporation of a specific substance into extracellular vesicles can be achieved by culturing cells genetically engineered to produce recombinant extracellular vesicles in a cell culture medium in which a drug is dissolved, or by placing the isolated recombinant extracellular vesicles in a solvent in which a therapeutic agent for a specific disease is dissolved and subjecting them to ultrasonification to reconstruct the extracellular vesicles. When selectively loading a drug into extracellular vesicles, the isolated extracellular vesicles can be simply mixed with the drug in an appropriate solvent or medium and stirred for an appropriate amount of time (Sun et al., Mol. Ther. 18(9): 1606-1614, 2010). In the case of a hydrophilic drug such as nucleic acids, they can be incorporated into extracellular vesicles using electroporation.

[0010] Meanwhile, extracellular vesicles have the advantage of being safe because they do not induce immune responses and have a very low risk of tumorigenesis because they cannot self-replicate, unlike cells. In particular, extracellular vesicles secreted from mesenchymal stem cells have the characteristic of effectively reaching tumor cells and being less toxic even after repeated administration. In addition, extracellular vesicles are much smaller than the cell size of 10,000 nm to 20,000 nm, so they can be administered without the risk of embolism, a potential risk factor for cell therapeutic agents. Accordingly, attempts are being made to apply the extracellular vesicles to cancer treatment.

[0011] Therefore, there is an urgent need to develop a method for preparing extracellular vesicles that can effectively apply the characteristics of these extracellular vesicles to anticancer agents.

## DETAILED DESCRIPTION OF INVENTION

### Technical Problem

[0012] Accordingly, the present inventors conducted research to develop extracellular vesicles as an anticancer agent. As a result, the present inventors found that extracellular vesicles isolated from cells treated with zymosan, Poly I:C, and monophosphoryl lipid A (MPLA) comprise various miRNAs, mRNAs, and cytokines and exhibit excellent anticancer effects. Based on the above, the present inventors completed the present invention.

### Solution to Problem

[0013] In order to achieve the above object, in one aspect of the present invention, there is provided an extracellular vesicle comprising various miRNAs, mRNAs, and cytokines.

[0014] In another aspect of the present invention, there is provided an extracellular vesicle isolated from a human-derived cell treated with zymosan, Poly I:C, and monophosphoryl lipid A (MPLA).

[0015] In another aspect of the present invention, there is provided a pharmaceutical composition for preventing or treating cancer, comprising the extracellular vesicle as an active ingredient.

[0016] In another aspect of the present invention, there is provided a pharmaceutical composition for combination administration for preventing or treating cancer, comprising the extracellular vesicle and an immune anticancer agent as active ingredients.

### Effects of Invention

[0017] The extracellular vesicles isolated from cells treated with zymosan, Poly I:C, and monophosphoryl lipid A (MPLA) comprise various miRNAs, mRNAs, and cytokines related to anticancer effects, and have tumor growth inhibitory effects. In addition, the extracellular vesicles exhibit a synergistic effect in cancer treatment when administered in combination with an immune checkpoint inhibitor. Therefore, a pharmaceutical composition for treating cancer, comprising the extracellular vesicles as an active ingredient can be effectively utilized in the prevention or treatment of various cancers.

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## Description

### BRIEF DESCRIPTION OF DRAWINGS

[0018] FIG. 1 is a graph showing the results obtained by measuring the number of particles by size of the exosomes isolated from mesenchymal stem cells treated with the chemical substance cocktail of the present invention (CHEM Exo) and the exosomes isolated from untreated mesenchymal stem cells (CTRL Exo) using an NTA equipment.

[0019] FIG. 2 shows the result obtained by performing Western blot to confirm the expression pattern of extracellular vesicle-specific markers of the exosomes isolated from mesenchymal stem cells treated with the chemical substance cocktail of the present invention (CHEM Exo).

[0020] FIG. 3 shows the result obtained by performing ELISA to analyze cytokines present in the exosomes isolated from mesenchymal stem cells treated with the chemical substance cocktail of the present invention (CHEM Exo).

[0021] FIG. 4 shows the result obtained by performing microarray to confirm miRNAs with a specifically increased expression level in the exosomes isolated from mesenchymal stem cells treated with the chemical substance cocktail of the present invention (CHEM Exo).

[0022] FIG. 5 shows the results obtained by measuring the amount of nitric oxide production in a mouse leukemia cell line (RAW 264.7) following the treatment of the exosomes isolated from mesenchymal stem cells treated with the chemical substance cocktail of the present invention. The data represent the mean±standard error of three samples, and \*\*\* $p < 0.001$  is a value derived from one-way ANOVA after multiple comparisons with Student's two-tailed t test.

[0023] FIG. 6 shows the result obtained by performing RT-qPCR to measure the intensity of expression of proinflammatory cytokines and inflammation-related proteins in a mouse leukemia cell line (RAW 264.7) following the treatment of the exosomes isolated from mesenchymal stem cells treated with the chemical substance cocktail of the present invention. The data represent the mean±standard error of three samples, and \*\*\* $p < 0.001$  is a value derived from one-way ANOVA after multiple comparisons with Student's two-tailed t test.

[0024] FIG. 7 shows the results obtained by measuring the change in tumor volume by group in a mouse colorectal cancer model following the administration of the exosomes isolated from mesenchymal stem cells or mesenchymal stem cells differentiated from induced pluripotent stem cells treated with the chemical substance cocktail of the present invention (IEP-01 or IEP-01-i) alone or the administration in combination with an anti-PD-1 antibody. The error bars in the graph represent the Standard Error Mean (SEM).

[0025] FIG. 8 shows the results obtained by measuring the change in tumor volume by subject in a mouse colorectal cancer model following the administration of the exosomes isolated from mesenchymal stem cells or mesenchymal stem cells differentiated from induced pluripotent stem cells treated with the chemical substance cocktail of the present invention (IEP-01 or IEP-01-i) alone or the administration in combination with an anti-PD-1 antibody.

[0026] FIG. 9 shows the results obtained by measuring the body weight by subject in a mouse colorectal cancer model following the administration of the exosomes isolated from mesenchymal stem cells or mesenchymal stem cells differentiated from induced pluripotent stem cells treated with the chemical substance cocktail of the present invention (IEP-01 or IEP-01-i) alone or the administration in combination with an anti-PD-1 antibody.

[0027] FIG. 10 shows the results obtained by measuring the change in tumor weight by group in a mouse colorectal cancer model following the administration of the exosomes isolated from mesenchymal stem cells or mesenchymal stem cells differentiated from induced pluripotent stem cells treated with the chemical substance cocktail of the present invention (IEP-01 or IEP-01-i) alone or the administration in combination with an anti-PD-1 antibody. The error bars in the graph represent the Standard Error Mean (SEM). \* $p < 0.05$ , \*\* $p < 0.01$  are values derived from one-way

ANOVA after multiple comparisons with Student's one-tailed t test

[0028] FIG. **11** is a photograph of extracted tumors by subject in a mouse colorectal cancer model following the administration of the exosomes isolated from mesenchymal stem cells or mesenchymal stem cells differentiated from induced pluripotent stem cells treated with the chemical substance cocktail of the present invention (IEP-01 or IEP-01-i) alone or the administration in combination with an anti-PD-1 antibody.

[0029] FIG. **12** shows the results obtained by measuring the level of IL-6 mRNA expression in a mouse leukemia cell line (RAW 264.7) by qRT-PCR after treatment with the exosomes isolated from mesenchymal stem cells treated with the chemical substance cocktail of the present invention alone (IEP-01) or after treatment with POLY I:C alone.

[0030] FIG. **13a** is a graph showing the level of IL-6 expression after treating Raw264.7 cells with exosomes obtained from untreated WJ-MSCs (naive), exosomes obtained from WJ-MSCs treated with 3-chem or 2-chem (IEP-01 or IEP-01'), or LPS for 24 hours.

[0031] FIG. **13b** is a graph showing the level of IL-1 $\beta$  expression after treating Raw264.7 cells with exosomes obtained from untreated WJ-MSCs (naive), exosomes obtained from WJ-MSCs treated with 3-chem or 2-chem (IEP-01 or IEP-01'), or LPS for 24 hours.

[0032] FIG. **13c** is a graph showing the level of iNOS2 expression after treating Raw264.7 cells with exosomes obtained from untreated WJ-MSCs (naive), exosomes obtained from WJ-MSCs treated with 3-chem or 2-chem (IEP-01 or IEP-01'), or LPS for 24 hours.

[0033] FIG. **13d** is a graph showing the level of IL-6 expression after treating Raw264.7 cells with exosomes obtained from untreated ciMSCs (naive), exosomes obtained from ciMSCs treated with 3-chem or 2-chem (IEP-01i or IEP-01i'), or LPS for 24 hours.

[0034] FIG. **13e** is a graph showing the level of iNOS2 expression after treating Raw264.7 cells with exosomes obtained from untreated ciMSCs (naive), exosomes obtained from ciMSCs treated with 3-chem or 2-chem (IEP-01i or IEP-01i'), or LPS for 24 hours.

[0035] FIG. **13f** is a graph showing the level of IL-6 expression after treating Raw264.7 cells with exosomes obtained from untreated HEK293T cell line (naive), exosomes obtained from HEK293T cell line treated with 3-chem or 2-chem (IEP-01h or IEP-01h'), or LPS for 24 hours.

[0036] FIG. **13g** is a graph showing the level of IL-1 $\beta$  expression after treating Raw264.7 cells with exosomes obtained from untreated HEK293T cell line (naive), exosomes obtained from HEK293T cell line treated with 3-chem or 2-chem (IEP-01h or IEP-01h'), or LPS for 24 hours.

[0037] FIG. **14a** is a graph showing the degree of uptake of ciMSC-derived exosomes by PBMC and immune cell types.

[0038] FIG. **14b** is a graph showing the degree of uptake of ciMSC-derived exosomes by PBMC and immune cell types as measured by PKH67 MFI.

[0039] FIG. **14c** is a graph showing the degree of each immune cell that was positive for PKH67 as %.

[0040] FIG. **15a** shows the results of flow cytometry analysis for the degree of macrophage activation as measured by the level of CD80 expression after treating Raw264.7 cells with IEP-01i'.

[0041] FIG. **15b** is a graph showing the degree of macrophage activation as measured by the level of CD80 expression after treating Raw264.7 cells with IEP-01i'.

[0042] FIG. **15c** is a graph showing the results obtained by confirming the expression level of CD80, an M1 macrophage marker, by flow cytometry analysis when Raw264.7 cells were treated with IL-4 to induce differentiation into M2 macrophages and then treated with IEP-01i'.

[0043] FIG. **15d** is a graph showing the results obtained by confirming the expression level of CD80, an M1 macrophage marker, when Raw264.7 cells were treated with IL-4 to induce differentiation into M2 macrophages and then treated with IEP-01i'.

[0044] FIG. **16a** is a graph showing the ratio of dendritic cells in each experimental group as a ratio of CD11c+ cells.

[0045] FIG. **16b** shows that PKH-positive dendritic cells were observed in dendritic cells treated

with PKH-labeled exosomes.

[0046] FIG. **16c** confirms PKH-positive dendritic cells in dendritic cells treated with PKH-labeled naive exosomes.

[0047] FIG. **16d** confirms PKH-positive dendritic cells in dendritic cells treated with PKH-labeled IEP-01i'.

[0048] FIG. **16e** is a graph showing the expression level of CD80, an activation marker of dendritic cells, in each experimental group.

[0049] FIG. **16f** is a graph showing the expression level of CD80, an activation marker of dendritic cells, and the PKH-positive ratio in each experimental group.

[0050] FIG. **16g** is a graph showing the expression level of CD86, an activation marker of dendritic cells, in each experimental group.

[0051] FIG. **16h** is a graph showing the expression level of CD86, an activation marker of dendritic cells, and the PKH-positive ratio in each experimental group.

[0052] FIG. **16i** is a graph showing the expression level of MHC class I of dendritic cells in each experimental group.

[0053] FIG. **16j** is a graph showing the expression level of MHC class I of dendritic cells and the PKH-positive ratio in each experimental group.

[0054] FIG. **16k** is a graph showing the expression level of MHC class II of dendritic cells in each experimental group.

[0055] FIG. **16l** is a graph showing the expression level of MHC class II of dendritic cells and the PKH-positive ratio in each experimental group.

[0056] FIG. **17a** is a graph showing the results obtained by analyzing IP-10 included in naive exosomes or IEP-01i' obtained from ciMSCs.

[0057] FIG. **17b** is a graph showing the results obtained by analyzing IL-6 included in naive exosomes or IEP-01i' obtained from ciMSCs.

[0058] FIG. **17c** is a graph showing the results obtained by analyzing MCP-1 included in naive exosomes or IEP-01i' obtained from ciMSCs.

[0059] FIG. **17d** is a graph showing the results obtained by analyzing IL-8 included in naive exosomes or IEP-01i' obtained from ciMSCs.

[0060] FIG. **17e** is a graph showing the results obtained by analyzing TGF- $\beta$ 1 included in naive exosomes or IEP-01i' obtained from ciMSCs.

[0061] FIG. **18a** is a schematic diagram of an experiment to confirm the anticancer activity of IEP-01i' against colorectal cancer.

[0062] FIG. **18b** is a graph showing the change in tumor size when IEP-01i' was administered at a low dose (LD), a mid dose (MD), or a high dose (HD) to a CT26 colorectal cancer animal model.

[0063] FIG. **18c** is a graph showing the tumor growth of each subject in a group treated with PBS in a CT26 colorectal cancer animal model.

[0064] FIG. **18d** is a graph showing the tumor growth of each subject in a group treated with IEP-01i' at a low dose once every two days in a CT26 colorectal cancer animal model.

[0065] FIG. **18e** is a graph showing the tumor growth of each subject in a group treated with IEP-01i' at a mid dose once every two days in a CT26 colorectal cancer animal model.

[0066] FIG. **18f** is a graph showing the tumor growth of each subject in a group treated with IEP-01i' at a high dose once every two days in a CT26 colorectal cancer animal model.

[0067] FIG. **18g** is a graph showing the tumor growth of each subject in a group treated with IEP-01i' at a low dose every day in a CT26 colorectal cancer animal model.

[0068] FIG. **18h** is a graph showing the tumor growth of each subject in a group treated with IEP-01i' at a mid dose every day in a CT26 colorectal cancer animal model.

[0069] FIG. **18i** is a graph showing the tumor growth of each subject in a group treated with IEP-01i' at a high dose every day in a CT26 colorectal cancer animal model.

[0070] FIG. **19a** is a schematic diagram of an experiment to confirm the anticancer activity of IEP-

01 and IEP-01i against colorectal cancer.

[0071] FIG. **19b** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with an anti-PD-1 antibody, IEP-01, or IEP-01i alone or in combination in a CT26 colorectal cancer animal model.

[0072] FIG. **19c** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with PBS in a CT26 colorectal cancer animal model.

[0073] FIG. **19d** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with Ctrl Exo in a CT26 colorectal cancer animal model.

[0074] FIG. **19e** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with an anti-PD-1 antibody in a CT26 colorectal cancer animal model.

[0075] FIG. **19f** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with IEP-01 in a CT26 colorectal cancer animal model.

[0076] FIG. **19g** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with IEP-01 and an anti-PD-1 antibody in combination in a CT26 colorectal cancer animal model.

[0077] FIG. **19h** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with IEP-01i in a CT26 colorectal cancer animal model.

[0078] FIG. **19i** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with IEP-01i and an anti-PD-1 antibody in combination in a CT26 colorectal cancer animal model.

[0079] FIG. **20a** is a schematic diagram of an experiment to confirm the anticancer activity of IEP-01i in a B16F10 melanoma model.

[0080] FIG. **20b** is a graph showing the tumor size of B16F10 by date after treatment with an anti-PD-1 antibody or IEP-01i alone or in combination, respectively, three times a week for a total of two weeks in a B16F10 melanoma animal model.

[0081] FIG. **20c** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with PBS in a B16F10 melanoma animal model.

[0082] FIG. **20d** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with an anti-PD-1 antibody in a B16F10 melanoma animal model.

[0083] FIG. **20e** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with IEP-01i in a B16F10 melanoma animal model.

[0084] FIG. **20f** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with IEP-01i and an anti-PD-1 antibody in combination in a B16F10 melanoma animal model.

[0085] FIG. **21a** is a schematic diagram of an experiment to confirm the anticancer activity of IEP-01i' in a Pan02 pancreatic cancer animal model.

[0086] FIG. **21b** is a graph showing the results obtained by analyzing the tumor size by date after treatment with an anti-PD-1 antibody or IEP-01i' alone or in combination, respectively, three times a week for a total of two weeks in a Pan02 pancreatic cancer animal model.

[0087] FIG. **21c** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with the control group in a Pan02 pancreatic cancer animal model.

[0088] FIG. **21d** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with an anti-PD-1 antibody in a Pan02 pancreatic cancer animal model.

[0089] FIG. **21e** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with IEP-01i' in a Pan02 pancreatic cancer animal model.

[0090] FIG. **21f** is a graph showing the results obtained by analyzing the tumor size of each subject by date after treatment with IEP-01i' and an anti-PD-1 antibody in combination in a Pan02 pancreatic cancer animal model.

[0091] FIG. **22a** is a graph showing the results obtained by analyzing whether poly IC is detected in samples obtained before and after purifying the cell culture medium spiked with polyLC, using

HPLC.

[0092] FIG. 22b is a graph showing the results obtained by analyzing whether poly IC is detected in samples before and after the IEP-01 purification process, using HPLC.

[0093] FIG. 22c is a graph showing HPLC analysis results for whether poly IC is detected in IEP-01 or naive exosomes.

[0094] FIG. 23 is a heat map showing miRNAs that are specifically abundant in IEP-01i or IEP-01i' compared to naive exosomes by analyzing miRNAs included in naive exosomes (Ctrl Exo), IEP-01i (3 chem Exo), or IEP-01i' (2 chem Exo) using microarray.

#### MODE FOR CARRYING OUT THE INVENTION

##### Novel Extracellular Vesicles Isolated from Mesenchymal Stem Cells

[0095] In one aspect of the present invention, there is provided an extracellular vesicle isolated from a human-derived cell, wherein the extracellular vesicle [0096] i) comprises at least one miRNA selected from the group consisting of NovelmiRNA-67, hsa-miR-199a-3p, NovelmiRNA-298, hsa-miR-145-5p, NovelmiRNA-281, NovelmiRNA-109, hsa-miR-432-5p, NovelmiRNA-230, NovelmiRNA-88, hsa-miR-200b-3p, NovelmiRNA-169, hsa-miR-200a-3p, hsa-miR-487b-3p, hsa-miR-214-3p, NovelmiRNA-208, NovelmiRNA-164, NovelmiRNA-225, NovelmiRNA-174, hsa-miR-708-5p, hsa-miR-124-3p, NovelmiRNA-150, hsa-miR-135a-5p, NovelmiRNA-64, NovelmiRNA-92, NovelmiRNA-168, NovelmiRNA-22, NovelmiRNA-35, NovelmiRNA-161, hsa-miR-3940-3p, hsa-miR-219a-3p, hsa-miR-212-3p, NovelmiRNA-76, hsa-miR-323a-3p, hsa-miR-654-3p, hsa-miR-204-5p, hsa-miR-136-3p, NovelmiRNA-39, NovelmiRNA-175, hsa-miR-134-5p, hsa-miR-454-3p, NovelmiRNA-286, hsa-miR-542-3p, hsa-miR-433-3p, hsa-miR-574-5p, hsa-miR-6086, hsa-miR-4701-3p, hsa-miR-3149, hsa-miR-4743-5p, hsa-miR-8075, hsa-miR-6880-5p, hsa-miR-3148, hsa-miR-6780b-5p, hsa-miR-6846-5p, hsa-miR-320e, hsa-miR-32-3p, hsa-miR-642a-3p, hsa-miR-4505, hsa-miR-595, hsa-miR-4487, hsa-miR-4721, hsa-miR-7151-3p, hsa-miR-4484, hsa-miR-642b-3p, ENSG00000239154, hsa-miR-4706, hsa-miR-6126, hsa-miR-378h, hsa-miR-6849-5p, hsa-miR-7844-5p, hsa-miR-4535, hsa-miR-9-1, hsa-miR-4459, hsa-miR-4443, hsa-miR-6732-5p, hsa-miR-3064-5p, hsa-miR-3178, hsa-miR-3135b, hsa-miR-4717-3p, hsa-miR-206, hsa-miR-4529-3p, and hsa-miR-3613-5p, and [0097] ii) comprises at least one mRNA selected from the group consisting of MED13L, lnc-TOMM20L-1, lnc-CDK5R1-1, ETV3, SLC35G1, SMARCA4, ACAT2, ADAM12, ARFGEF1, CELF3, DNAJB5, lnc-WRNIP1-2, ACTL8, lnc-TM2D3-2, GCN1L1, LOC101929337, FAM217A, XLOC\_l2\_013467, SNHG4, NUDT13, MCTS1, ATG4D, NPEPL1, YEATS2, SPEN, GGT6, lnc-RP11-770J1.4.1-1, MIAT, lnc-HMCN1-2, RETN, lnc-EXT1-2, LIN54, C17orf74, SLC12A2, TTC28, ADRA2A, NLGN2, lnc-FBXO25-4, LOC100506603, LOC399886, RCC2, KIAA0040, HOXB9, JMJD8, LINC01146, lnc-GIT1-1, VRTN, NHP2, KIF26A, HAUS5, lnc-SOD2-2, APOL3, lnc-POLR2L-1, lnc-SUPT3H-1, lnc-XRN2-3, RTP2, TERT, TNFRSF12A, LOC645427, lnc-CTR9-5, LOC100507420, OPA3, XLOC\_l2\_014245, lnc-NR5A1-1, DMTN, SMPD4, TXLNB, SPRR1A, DISP1, LOC100130285, lnc-SLC17A9-1, lnc-SELV.1-1, XLOC\_l2\_000018, lnc-ROM1-2, lnc-RABEPK-1, lnc-RRP8-1, lnc-RP11-234B24.6.1-1, lnc-TMEM189-UBE2V1-2, LAMTOR2, BTK, lnc-BSND-1, C2orf71, lnc-C17orf63-2, lnc-CHGA-1, ABCD1, NCAN, C9orf106, AGAP2, EMC6, CD86, LOC100129175, lnc-PHYHD1-1, GIGYF2, RNF151, BAGE, ADRA2C, lnc-SLC43A1-1, ENTPD8, NYAP1, lnc-GLIPR1L1-1, CCNT2-AS1, NAV1, TBX21, GNAZ, DNMT3OS, PRKAG2-AS1, lnc-MFSD9-4, lnc-RPGRIP1-2, ABCC6, LRFN3, lnc-COL1A1-2, lnc-PPAPDC3-2, CTU1, lnc-PABPC4-1, LOC100507165, LOC102724861, SPTSSB, lnc-C20orf96-4, DCDC5, lnc-CRYL1-1, LOC100506114, SHARPIN, ASMTL-AS1, HMOX1, SHARPIN, LOC101928207, TRAIP, lnc-ANTXRL-2, LINC01135, BZRAP1, LOC101926983, WDR6, C19orf81, NAMA, LIM2, FAM155A-IT1, lnc-RPP30-2, ZNF264, MALAT1, HTT-AS, ZDHHC22, SEPHS2, ABTB1, lnc-RP11-791J7.2.1-1, CCDC28B, LOC284263, lnc-PPIC-2, CDC42BPB, C9orf173-AS1, C9orf139, FGD5, SP6, RCOR2, lnc-AC016251.1-2, lnc-PRKAA2-1, RANBP3, lnc-SH2B3-1, XLOC\_l2\_011901, CACNA1H, LINC01314, lnc-VAPB-1, ARHGAP23, ATG9A, GM2A,



XLOC\_12\_013547, lnc-CLC25A26-1, TMEM240, CLSTN3, ATG16L2, MMP11, TSSK3, lnc-SOX6-1, EDEM3, LOC101927210, C14orf169, EBLN2, lnc-XRN1-1, lnc-FTSJD2-1, MDFI, LOC102546226, SLC5A10, SENP7, lnc-C17orf97-4, ARNTL, lnc-RTL1-1, ZNF713, XLOC\_12\_005871, QSOX1, SP2-AS1, ARSA, PHF7, GS1-24F4.2, LRIT1, PLLP, SLC4A11, C11orf95, FAM110B, IER5, LINC01508, lnc-GTPBP8-1, LOC400958, WFDC21P, lnc-CPXM2-2, P2RX6, SLC22A7, lnc-PPP2R2C-1, lnc-GATA5-2, MARVELDI, lnc-TRIM41-3, PRSS8, SEMA6C, SLC48A1, RPL28, BGN, BZW1, lnc-C6orf221-2, SLC20A2, HYALP1, SNAR-G1, SEC22A, TIMM8B, ATAD1, FNDC7, B3GNT8, STARD7-AS1, TMEM100, LARP6, HELZ2, TMX2, lnc-EPHB6-1, PSMC2, lnc-LINC00273-1, TNFRSF25, BCL3, LOC101929450, LOC102724416, FLII, WAC, PPP6R1, LOC100507377, LRRFIP2, CCNT2-AS1, lnc-SCAMP5-1, BRD1, lnc-ELAVL4-3, CHRDL1, lnc-FUT8-1, lnc-DNAJC7-1, LINC01264, LMO2, LINC01197, LOC101927533, REP15, C1orf226, lnc-STEAP1B-1, SNORD3B-1, lnc-TMEM135-2, lnc-FAM160A1-1, GOLGA2P5, TMEM132C, GPX3, lnc-DCAF4L2-2, XLOC\_12\_014217, PPT1, RPA4, KCTD5, lnc-UQCRFS1-9, MTUS2-AS1, CHKA, TBC1D31, SNORD38A, lnc-RP11-322L20.1.1-7, RAD51, MEIOB, lnc-ADAMTS14-1, NOS2, and PCDHA13, and [0098] iii) comprises at least one cytokine selected from the group consisting of IL-6, IL-8, MCP-3, MCP-1, IP-10, IL-13, and RANTES.

[0099] The human-derived cell may include a cell, a cell line, or a stem cell derived from human tissue, but is not limited thereto.

[0100] The cell derived from human tissue may be a cell derived from the human heart, stomach, colorectal cancer, small intestine, lung, liver, kidney or uterus. In addition, the cell derived from human tissue may be a human-derived immortalized cell line.

[0101] The stem cell may be a mesenchymal stem cell, a hematopoietic stem cell, a neural stem cell, an embryonic stem cell, or an induced pluripotent stem cell. Specifically, the stem cell may be a mesenchymal stem cell.

[0102] As used herein, the term “mesenchymal stem cell (MSC)” refers to stem cells that exist in cartilage, bone tissue, adipose tissue, the stroma of bone marrow, and the like, differentiated from the mesoderm formed by division of a fertilized egg, and may include mesenchymal stem cells of animals, including mammals, for example humans. Mesenchymal stem cells maintain stemness and self-renewal and have the ability to differentiate into various cells, including chondrocytes, osteoblasts, muscle cells, and adipocytes, and can be extracted from Wharton's jelly, bone marrow, adipose tissue, umbilical cord blood, synovial membrane, bone tissue (trabecular bone), muscle, infrapatellar fat pad, peripheral blood, liver, teeth, hair follicles, and the like. Mesenchymal stem cells have immunomodulatory abilities that suppress the activity and proliferation of T lymphocytes and B lymphocytes, inhibit the activity of natural killer cells (NK cells), and regulate the functions of dendritic cells and macrophages, and are therefore cells capable of allotransplantation and xenotransplantation.

[0103] The mesenchymal stem cell may be derived from umbilical cord, umbilical cord blood, Wharton's jelly, bone marrow, fat, muscle, nerve, skin, amniotic membrane, or placenta, or may be differentiated from an induced pluripotent stem cell, but is not limited thereto. Specifically, the mesenchymal stem cell may be derived from Wharton's jelly.

[0104] As used herein, the term “derived cell”, for example, “derived mesenchymal stem cell”, etc., refers to a cell substantially isolated from the tissue from which the cell originates, for example, Wharton's jelly, or a cell differentiated from an induced stem cell.

[0105] As used herein, the term “induced pluripotent stem cell (iPSC)” refers to a cell that has been induced to have pluripotency like a stem cell by introducing a specific factor into a differentiated somatic cell to dedifferentiate.

[0106] As used herein, the term “differentiation” refers to a phenomenon in which structures or functions become specialized while cells divide and proliferate and grow, that is, cells, tissues, and the like of a living organism change in shape or function to perform their respective tasks.

[0107] As used herein, the term “extracellular vesicle” refers to a nano-sized particle naturally secreted by living cells, packaged in a lipid bilayer, and may be a concept that encompasses all vesicles (exosomes, microvesicles, multivesicles, and extracellular vesicle-like vesicles) that serve as information transfer mechanisms between cells and have a composition similar to that of extracellular vesicles.

[0108] As used herein, the term “isolated extracellular vesicle” refers to an extracellular vesicle substantially isolated from a cell from which an extracellular vesicle originates, for example, a mesenchymal stem cell.

[0109] In one embodiment, the extracellular vesicles may be isolated from a culture solution of mesenchymal stem cells derived from Wharton's jelly.

[0110] In one embodiment, the extracellular vesicles may be isolated from a culture solution of mesenchymal stem cells differentiated from induced pluripotent stem cells. The mesenchymal stem cells may be chemically induced to differentiate.

[0111] In one embodiment, the extracellular vesicles may be isolated from a culture solution of a HEK293T cell line.

[0112] In one embodiment, as a result of analyzing the miRNAs of extracellular vesicles isolated from mesenchymal stem cells cultured in a medium supplemented with at least one selected from the group consisting of zymosan, polyinosinic-polycytidylic acid (Poly I:C), and monophosphoryl lipid A (MPLA), the extracellular vesicle may comprise at least one miRNA selected from the group consisting of NovelmiRNA-67, hsa-miR-199a-3p, NovelmiRNA-298, hsa-miR-145-5p, NovelmiRNA-281, NovelmiRNA-109, hsa-miR-432-5p, NovelmiRNA-230, NovelmiRNA-88, hsa-miR-200b-3p, NovelmiRNA-169, hsa-miR-200a-3p, hsa-miR-487b-3p, hsa-miR-214-3p, NovelmiRNA-208, NovelmiRNA-164, NovelmiRNA-225, NovelmiRNA-174, hsa-miR-708-5p, hsa-miR-124-3p, NovelmiRNA-150, hsa-miR-135a-5p, NovelmiRNA-64, NovelmiRNA-92, NovelmiRNA-168, NovelmiRNA-22, NovelmiRNA-35, NovelmiRNA-161, hsa-miR-3940-3p, hsa-miR-219a-3p, hsa-miR-212-3p, NovelmiRNA-76, hsa-miR-323a-3p, hsa-miR-654-3p, hsa-miR-204-5p, hsa-miR-136-3p, NovelmiRNA-39, NovelmiRNA-175, hsa-miR-134-5p, hsa-miR-454-3p, NovelmiRNA-286, hsa-miR-542-3p, hsa-miR-433-3p, hsa-miR-574-5p, hsa-miR-6086, hsa-miR-4701-3p, hsa-miR-3149, hsa-miR-4743-5p, hsa-miR-8075, hsa-miR-6880-5p, hsa-miR-3148, hsa-miR-6780b-5p, hsa-miR-6846-5p, hsa-miR-320e, hsa-miR-32-3p, hsa-miR-642a-3p, hsa-miR-4505, hsa-miR-595, hsa-miR-4487, hsa-miR-4721, hsa-miR-7151-3p, hsa-miR-4484, hsa-miR-642b-3p, ENSG00000239154, hsa-miR-4706, hsa-miR-6126, hsa-miR-378h, hsa-miR-6849-5p, hsa-miR-7844-5p, hsa-miR-4535, hsa-miR-9-1, hsa-miR-4459, hsa-miR-4443, hsa-miR-6732-5p, hsa-miR-3064-5p, hsa-miR-3178, hsa-miR-3135b, hsa-miR-4717-3p, hsa-miR-206, hsa-miR-4529-3p, and hsa-miR-3613-5p. For example, the extracellular vesicle may comprise 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more or all miRNAs, among the above 43 types of miRNAs.

[0113] In one embodiment, as a result of analyzing the miRNAs of extracellular vesicles isolated from Wharton's jelly-derived mesenchymal stem cells cultured in a medium supplemented with at least one selected from the group consisting of zymosan, polyinosinic-polycytidylic acid (Poly I:C), and monophosphoryl lipid A (MPLA), the extracellular vesicle may comprise at least one miRNA selected from the group consisting of NovelmiRNA-67, hsa-miR-199a-3p, NovelmiRNA-298, hsa-miR-145-5p, NovelmiRNA-281, NovelmiRNA-109, hsa-miR-432-5p, NovelmiRNA-230, NovelmiRNA-88, hsa-miR-200b-3p, NovelmiRNA-169, hsa-miR-200a-3p, hsa-miR-487b-3p, hsa-miR-214-3p, NovelmiRNA-208, NovelmiRNA-164, NovelmiRNA-225, NovelmiRNA-174, hsa-miR-708-5p, hsa-miR-124-3p, NovelmiRNA-150, hsa-miR-135a-5p, NovelmiRNA-64, NovelmiRNA-92, NovelmiRNA-168, NovelmiRNA-22, NovelmiRNA-35, NovelmiRNA-161, hsa-miR-3940-3p, hsa-miR-219a-3p, hsa-miR-212-3p, NovelmiRNA-76, hsa-miR-323a-3p, hsa-miR-654-3p, hsa-miR-204-5p, hsa-miR-136-3p, NovelmiRNA-39, NovelmiRNA-175, hsa-miR-134-5p,

hsa-miR-454-3p, NovelmiRNA-286, hsa-miR-542-3p, and hsa-miR-433-3p. In addition, it may significantly comprise the miRNAs compared to the comparative exosomes that have not been treated with chemical substances.

[0114] hsa-miR-199a-3p and hsa-miR-200b-3p can inhibit cell proliferation and induce apoptosis; hsa-miR-145-5p, hsa-miR-432-5p and hsa-miR-200a-3p can inhibit cell migration and invasion; hsa-miR-214-3p and hsa-miR-708-5p can inhibit cell proliferation and cell cycle progression; hsa-miR-487b-3p can inhibit chemoresistance and metastasis of osteosarcoma; hsa-miR-124-3p can function as a tumor suppressor; and hsa-miR-135a-5p can inhibit tumor invasion.

[0115] In one embodiment, as a result of analyzing the miRNAs of extracellular vesicles isolated from mesenchymal stem cells differentiated from induced pluripotent stem cells cultured in a medium supplemented with at least one selected from the group consisting of zymosan, polyinosinic-polycytidylic acid (Poly I:C), and monophosphoryl lipid A (MPLA), the extracellular vesicle may comprise at least one miRNA selected from the group consisting of hsa-miR-574-5p, hsa-miR-6086, hsa-miR-4701-3p, hsa-miR-3149, hsa-miR-4743-5p, hsa-miR-8075, hsa-miR-6880-5p, hsa-miR-3148, hsa-miR-6780b-5p, hsa-miR-6846-5p, hsa-miR-320e, hsa-miR-32-3p, hsa-miR-642a-3p, hsa-miR-4505, hsa-miR-595, hsa-miR-4487, hsa-miR-4721, hsa-miR-7151-3p, hsa-miR-4484, hsa-miR-642b-3p, ENSG00000239154, hsa-miR-4706, hsa-miR-6126, hsa-miR-378h, hsa-miR-6849-5p, hsa-miR-7844-5p, hsa-miR-4535, hsa-miR-9-1, hsa-miR-4459, hsa-miR-4443, hsa-miR-6732-5p, hsa-miR-3064-5p, hsa-miR-3178, hsa-miR-3135b, hsa-miR-4717-3p, hsa-miR-206, hsa-miR-4529-3p, and hsa-miR-3613-5p. In addition, it may significantly comprise the miRNAs compared to the comparative exosomes that have not been treated with chemical substances.

[0116] In another embodiment, as a result of analyzing the mRNAs of extracellular vesicles isolated from mesenchymal stem cells cultured in a medium supplemented with at least one selected from the group consisting of zymosan, polyinosinic-polycytidylic acid (Poly I:C), and monophosphoryl lipid A (MPLA), the extracellular vesicle may comprise at least one mRNA selected from the group consisting of MED13L, lnc-TOMM20L-1, lnc-CDK5R1-1, ETV3, SLC35G1, SMARCA4, ACAT2, ADAM12, ARFGEF1, CELF3, DNAJB5, lnc-WRNIP1-2, ACTL8, lnc-TM2D3-2, GCN1L1, LOC101929337, FAM217A, XLOC\_l2\_013467, SNHG4, NUDT13, MCTS1, ATG4D, NPEPL1, YEATS2, SPEN, GGT6, lnc-RP11-770J1.4.1-1, MIAT, lnc-HMCN1-2, RETN, lnc-EXT1-2, LIN54, C17orf74, SLC12A2, TTC28, ADRA2A, NLGN2, lnc-FBXO25-4, LOC100506603, LOC399886, RCC2, KIAA0040, HOXB9, JMJD8, LINC01146, lnc-GIT1-1, VRTN, NHP2, KIF26A, HAUS5, lnc-SOD2-2, APOL3, lnc-POLR2L-1, lnc-SUPT3H-1, lnc-XRN2-3, RTP2, TERT, TNFRSF12A, LOC645427, lnc-CTR9-5, LOC100507420, OPA3, XLOC\_l2\_014245, lnc-NR5A1-1, DMTN, SMPD4, TXLNB, SPRR1A, DISP1, LOC100130285, lnc-SLC17A9-1, lnc-SELV.1-1, XLOC\_l2\_000018, lnc-ROM1-2, lnc-RABEPK-1, lnc-RRP8-1, lnc-RP11-234B24.6.1-1, lnc-TMEM189-UBE2V1-2, LAMTOR2, BTK, lnc-BSND-1, C2orf71, lnc-C17orf63-2, lnc-CHGA-1, ABCD1, NCAN, C9orf106, AGAP2, EMC6, CD86, LOC100129175, lnc-PHYHD1-1, GIGYF2, RNF151, BAGE, ADRA2C, lnc-SLC43A1-1, ENTPD8, NYAP1, lnc-GLIPR1L1-1, CCNT2-AS1, NAV1, TBX21, GNAZ, DNMT3OS, PRKAG2-AS1, lnc-MFSD9-4, lnc-RPGRIP1-2, ABCC6, LRFN3, lnc-COL1A1-2, lnc-PPAPDC3-2, CTU1, lnc-PABPC4-1, LOC100507165, LOC102724861, SPTSSB, lnc-C20orf96-4, DCDC5, lnc-CRYL1-1, LOC100506114, SHARPIN, ASMTL-AS1, HMOX1, SHARPIN, LOC101928207, TRAIP, lnc-ANTXRL-2, LINC01135, BZRAP1, LOC101926983, WDR6, C19orf81, NAMA, LIM2, FAM155A-IT1, lnc-RPP30-2, ZNF264, MALAT1, HTT-AS, ZDHHC22, SEPHS2, ABTB1, lnc-RP11-791J7.2.1-1, CCDC28B, LOC284263, lnc-PPIC-2, CDC42BPB, C9orf173-AS1, C9orf139, FGD5, SP6, RCOR2, lnc-AC016251.1-2, lnc-PRKAA2-1, RANBP3, lnc-SH2B3-1, XLOC\_l2\_011901, CACNA1H, LINC01314, lnc-VAPB-1, ARHGAP23, ATG9A, GM2A, XLOC\_l2\_013547, lnc-SLC25A26-1, TMEM240, CLSTN3, ATG16L2, MMP11, TSSK3, lnc-SOX6-1, EDEM3, LOC101927210, C14orf169, EBLN2, lnc-XRN1-1, lnc-FTSJD2-1, MDFI, LOC102546226, SLC5A10, SENP7, lnc-C17orf97-4, ARNTL, lnc-RTL1-1, ZNF713,

XLOC\_12\_005871, QSOX1, SP2-AS1, ARSA, PHF7, GS1-24F4.2, LRIT1, PLLP, SLC4A11, C11orf95, FAM110B, IER5, LINC01508, lnc-GTPBP8-1, LOC400958, WFDC21P, lnc-CPXM2-2, P2RX6, SLC22A7, lnc-PPP2R2C-1, lnc-GATA5-2, MARVELDI, lnc-TRIM41-3, PRSS8, SEMA6C, SLC48A1, RPL28, BGN, BZW1, lnc-C6orf221-2, SLC20A2, HYALP1, SNAR-G1, SEC22A, TIMM8B, ATAD1, FNDC7, B3GNT8, STARD7-AS1, TMEM100, LARP6, HELZ2, TMX2, lnc-EPHB6-1, PSMC2, lnc-LINC00273-1, TNFRSF25, BCL3, LOC101929450, LOC102724416, FLII, WAC, PPP6R1, LOC100507377, LRRFIP2, CCNT2-AS1, lnc-SCAMP5-1, BRD1, lnc-ELAVL4-3, CHRDL1, lnc-FUT8-1, lnc-DNAJC7-1, LINC01264, LMO2, LINC01197, LOC101927533, REP15, C1orf226, lnc-STEAP1B-1, SNORD3B-1, lnc-TMEM135-2, lnc-FAM160A1-1, GOLGA2P5, TMEM132C, GPX3, lnc-DCAF4L2-2, XLOC\_12\_014217, PPT1, RPA4, KCTD5, lnc-UQCRFS1-9, MTUS2-AS1, CHKA, TBC1D31, SNORD38A, lnc-RP11-322L20.1.1-7, RAD51, MEIOB, lnc-ADAMTS14-1, NOS2, and PCDHA13. For example, the extracellular vesicle may comprise 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more or all mRNAs, among the above 275 types of mRNAs.

[0117] In another embodiment, as a result of analyzing the cytokines of extracellular vesicles isolated from mesenchymal stem cells cultured in a medium supplemented with at least one selected from the group consisting of zymosan, polyinosinic-polycytidylic acid (Poly I:C), and monophosphoryl lipid A (MPLA), the extracellular vesicle may comprise at least one cytokine selected from the group consisting of IL-6, IL-8, MCP-3, MCP-1, IP-10, and RANTES. For example, the extracellular vesicle may comprise 2 or more, 3 or more, 4 or more, 5 or more or all cytokines, among the above 6 types of cytokines.

[0118] The extracellular vesicle may comprise at least one selected from the group consisting of zymosan, Poly I:C, and monophosphoryl lipid A (MPLA).

[0119] As used herein, the term “zymosan” is a protein and carbohydrate complex obtained from yeast cell walls, and refers to a glucan in which glucose units linked by  $\beta$ -1,3-glycosidic bonds are repeated. Zymosan may be a compound represented by the following Formula 1.

##STR00001##

[0120] As used herein, the term “polyinosinic-polycytidylic acid (Poly I:C)” refers to an immunostimulant. Poly I:C is known to interact with toll-like receptor 3 (TLR3) expressed on the endosomal membrane of macrophages and dendritic cells, and may be a compound represented by the following Formula 2.

##STR00002##

[0121] As used herein, the term “monophosphoryl lipid A (MPLA)” is a modified form of lipid A and refers to an agonist of toll-like receptor 4. Monophosphoryl lipid A (MPLA) is derived from the cell wall of non-pathogenic *Salmonella* and is known to have immunostimulating activity and may be a compound represented by the following Formula 3.

##STR00003##

[0122] The extracellular vesicle i) may be positive for at least one marker selected from the group consisting of CD9, CD63, CD81, and TSG101, and ii) may be negative for at least one marker selected from the group consisting of GM130 and Calnexin.

[0123] As used herein, the term “positive” may mean, with respect to a specific marker, that the marker is present in a greater amount or at a higher concentration compared to a reference control. That is, the extracellular vesicle according to the present invention is positive for a marker if the marker is present on the surface or inside the extracellular vesicles and the extracellular vesicles can be distinguished from one or more non-extracellular vesicles or other extracellular vesicles using the marker. For example, the extracellular vesicle of the present invention can be detected with an antibody specific for CD81, an extracellular vesicle-specific surface antigen, and if the

signal intensity from this antibody is detectably greater than that of the control group (for example, background value), the extracellular vesicle is “CD81.sup.+ (CD81 positive)”.

[0124] As used herein, the term “negative” means that the marker is not detectable compared to background values even when using a specific antibody. For example, if the extracellular vesicle of the present invention cannot be detectably labeled with an antibody specific for GM130, the extracellular vesicle is “GM130.sup.– (GM130 negative)”.

[0125] The above immunological characteristics can be determined by conventional methods known in the art to which the present invention pertains. For example, various methods such as flow cytometry, immunocytochemical staining, Western blot, or RT-PCR can be used.

[0126] In another aspect of the present invention, there is provided an extracellular vesicle isolated from mesenchymal stem cells treated with at least one selected from the group consisting of zymosan, Poly I:C, and monophosphoryl lipid A (MPLA).

[0127] The extracellular vesicle i) may comprise at least one miRNA selected from the group consisting of NovelmiRNA-67, hsa-miR-199a-3p, NovelmiRNA-298, hsa-miR-145-5p, NovelmiRNA-281, NovelmiRNA-109, hsa-miR-432-5p, NovelmiRNA-230, NovelmiRNA-88, hsa-miR-200b-3p, NovelmiRNA-169, hsa-miR-200a-3p, hsa-miR-487b-3p, hsa-miR-214-3p, NovelmiRNA-208, NovelmiRNA-164, NovelmiRNA-225, NovelmiRNA-174, hsa-miR-708-5p, hsa-miR-124-3p, NovelmiRNA-150, hsa-miR-135a-5p, NovelmiRNA-64, NovelmiRNA-92, NovelmiRNA-168, NovelmiRNA-22, NovelmiRNA-35, NovelmiRNA-161, hsa-miR-3940-3p, hsa-miR-219a-3p, hsa-miR-212-3p, NovelmiRNA-76, hsa-miR-323a-3p, hsa-miR-654-3p, hsa-miR-204-5p, hsa-miR-136-3p, NovelmiRNA-39, NovelmiRNA-175, hsa-miR-134-5p, hsa-miR-454-3p, NovelmiRNA-286, hsa-miR-542-3p, hsa-miR-433-3p, hsa-miR-574-5p, hsa-miR-6086, hsa-miR-4701-3p, hsa-miR-3149, hsa-miR-4743-5p, hsa-miR-8075, hsa-miR-6880-5p, hsa-miR-3148, hsa-miR-6780b-5p, hsa-miR-6846-5p, hsa-miR-320e, hsa-miR-32-3p, hsa-miR-642a-3p, hsa-miR-4505, hsa-miR-595, hsa-miR-4487, hsa-miR-4721, hsa-miR-7151-3p, hsa-miR-4484, hsa-miR-642b-3p, ENSG00000239154, hsa-miR-4706, hsa-miR-6126, hsa-miR-378h, hsa-miR-6849-5p, hsa-miR-7844-5p, hsa-miR-4535, hsa-miR-9-1, hsa-miR-4459, hsa-miR-4443, hsa-miR-6732-5p, hsa-miR-3064-5p, hsa-miR-3178, hsa-miR-3135b, hsa-miR-4717-3p, hsa-miR-206, hsa-miR-4529-3p, and hsa-miR-3613-5p, and [0128] ii) may comprise at least one mRNA selected from the group consisting of MED13L, lnc-TOMM20L-1, lnc-CDK5R1-1, ETV3, SLC35G1, SMARCA4, ACAT2, ADAM12, ARFGEF1, CELF3, DNAJB5, lnc-WRNIP1-2, ACTL8, lnc-TM2D3-2, GCN1L1, LOC101929337, FAM217A, XLOC\_l2\_013467, SNHG4, NUDT13, MCTS1, ATG4D, NPEPL1, YEATS2, SPEN, GGT6, lnc-RP11-770J1.4.1-1, MIAT, lnc-HMCN1-2, RETN, lnc-EXT1-2, LIN54, C17orf74, SLC12A2, TTC28, ADRA2A, NLGN2, lnc-FBXO25-4, LOC100506603, LOC399886, RCC2, KIAA0040, HOXB9, JMJD8, LINC01146, lnc-GIT1-1, VRTN, NHP2, KIF26A, HAUS5, lnc-SOD2-2, APOL3, lnc-POLR2L-1, lnc-SUPT3H-1, lnc-XRN2-3, RTP2, TERT, TNFRSF12A, LOC645427, lnc-CTR9-5, LOC100507420, OPA3, XLOC\_l2\_014245, lnc-NR5A1-1, DMTN, SMPD4, TXLNB, SPRR1A, DISP1, LOC100130285, lnc-SLC17A9-1, lnc-SELV.1-1, XLOC\_l2\_000018, lnc-ROM1-2, lnc-RABEPK-1, lnc-RRP8-1, lnc-RP11-234B24.6.1-1, lnc-TMEM189-UBE2V1-2, LAMTOR2, BTK, lnc-BSND-1, C2orf71, lnc-C17orf63-2, lnc-CHGA-1, ABCD1, NCAN, C9orf106, AGAP2, EMC6, CD86, LOC100129175, lnc-PHYHD1-1, GIGYF2, RNF151, BAGE, ADRA2C, lnc-SLC43A1-1, ENTPD8, NYAP1, lnc-GLIPR1L1-1, CCNT2-AS1, NAV1, TBX21, GNAZ, DNMT3OS, PRKAG2-AS1, lnc-MFSD9-4, lnc-RPGRIP1-2, ABCC6, LRFN3, lnc-COL1A1-2, lnc-PPAPDC3-2, CTU1, lnc-PABPC4-1, LOC100507165, LOC102724861, SPTSSB, lnc-C20orf96-4, DCDC5, lnc-CRYL1-1, LOC100506114, SHARPIN, ASMTL-AS1, HMOX1, SHARPIN, LOC101928207, TRAIP, lnc-ANTXRL-2, LINC01135, BZRAP1, LOC101926983, WDR6, C19orf81, NAMA, LIM2, FAM155A-IT1, lnc-RPP30-2, ZNF264, MALAT1, HTT-AS, ZDHHC22, SEPHS2, ABTB1, lnc-RP11-791J7.2.1-1, CCDC28B, LOC284263, lnc-PPIC-2, CDC42BPB, C9orf173-AS1, C9orf139, FGD5, SP6, RCOR2, lnc-AC016251.1-2, lnc-PRKAA2-1, RANBP3, lnc-SH2B3-1,

XLOC\_12\_011901, CACNA1H, LINC01314, lnc-VAPB-1, ARHGAP23, ATG9A, GM2A, XLOC\_12\_013547, lnc-SLC25A26-1, TMEM240, CLSTN3, ATG16L2, MMP11, TSSK3, lnc-SOX6-1, EDEM3, LOC101927210, C14orf169, EBLN2, lnc-XRN1-1, lnc-FTSJD2-1, MDFI, LOC102546226, SLC5A10, SENP7, lnc-C17orf97-4, ARNTL, lnc-RTL1-1, ZNF713, XLOC\_12\_005871, QSOX1, SP2-AS1, ARSA, PHF7, GS1-24F4.2, LRIT1, PLLP, SLC4A11, C11orf95, FAM110B, IER5, LINC01508, lnc-GTPBP8-1, LOC400958, WFDC21P, lnc-CPXM2-2, P2RX6, SLC22A7, lnc-PPP2R2C-1, lnc-GATA5-2, MARVELDI, lnc-TRIM41-3, PRSS8, SEMA6C, SLC48A1, RPL28, BGN, BZW1, lnc-C6orf221-2, SLC20A2, HYALP1, SNAR-G1, SEC22A, TIMM8B, ATAD1, FNDC7, B3GNT8, STARD7-AS1, TMEM100, LARP6, HELZ2, TMX2, lnc-EPHB6-1, PSMC2, lnc-LINC00273-1, TNFRSF25, BCL3, LOC101929450, LOC102724416, FLII, WAC, PPP6R1, LOC100507377, LRRFIP2, CCNT2-AS1, lnc-SCAMP5-1, BRD1, lnc-ELAVL4-3, CHRDL1, lnc-FUT8-1, lnc-DNAJC7-1, LINC01264, LMO2, LINC01197, LOC101927533, REP15, C1orf226, lnc-STEAP1B-1, SNORD3B-1, lnc-TMEM135-2, lnc-FAM160A1-1, GOLGA2P5, TMEM132C, GPX3, lnc-DCAF4L2-2, XLOC\_12\_014217, PPT1, RPA4, KCTD5, lnc-UQCRFS1-9, MTUS2-AS1, CHKA, TBC1D31, SNORD38A, lnc-RP11-322L20.1.1-7, RAD51, MEIOB, lnc-ADAMTS14-1, NOS2, and PCDHA13, and [0129] iii) may comprise at least one cytokine selected from the group consisting of IL-6, IL-8, MCP-3, MCP-1, IP-10, IL-13, and RANTES.

[0130] In one embodiment, the extracellular vesicle may be treated with Poly I:C and monophosphoryl lipid A (MPLA); or zymosan, Poly I:C, and monophosphoryl lipid A (MPLA).

[0131] In one embodiment, the extracellular vesicle may be treated with Poly I:C and monophosphoryl lipid A (MPLA); or zymosan, Poly I:C, and monophosphoryl lipid A (MPLA).

[0132] The extracellular vesicle is as described above.

Pharmaceutical Use of Novel Extracellular Vesicles Isolated from Mesenchymal Stem Cells

[0133] In another aspect of the present invention, there is provided a pharmaceutical composition for treating or preventing cancer, comprising the extracellular vesicle as an active ingredient.

[0134] The pharmaceutical composition for preventing or treating cancer, comprising as an active ingredient the extracellular vesicle isolated from mesenchymal stem cells treated with at least one selected from the group consisting of zymosan, Poly I:C, and monophosphoryl lipid A (MPLA), according to the present invention, can increase the efficacy of cancer treatment and/or prevention.

[0135] The extracellular vesicle is as described above.

[0136] In one embodiment, the immune stimulating activity and tumor growth inhibitory effect of the extracellular vesicles isolated from Wharton's jelly-derived mesenchymal stem cells treated with zymosan, Poly I:C, and monophosphoryl lipid A (MPLA) against cancer cells can be confirmed.

[0137] In another embodiment, the tumor growth inhibitory effect of the extracellular vesicles isolated after culturing mesenchymal stem cells differentiated from induced pluripotent stem cells by treating them with zymosan, Poly I:C, and monophosphoryl lipid A (MPLA) can be confirmed.

[0138] The cancer may be any one selected from the group consisting of gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myeloid leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer, and lymphoma, but is not limited thereto.

[0139] The preferred dosage of the pharmaceutical composition varies depending on the patient's condition and body weight, the severity of the disease, the drug form, and the route and period of administration, but can be appropriately selected by those of ordinary skill in the art. In the pharmaceutical composition for preventing or treating cancer of the present invention, the active ingredient may be included in any amount (effective amount) depending on the use, formulation, compounding purpose, and the like, as long as it can exhibit anticancer activity. The typical effective amount of extracellular vesicles will be determined within the range of 0.001 wt % to 20.0

wt % based on the total weight of the composition. Here, the “effective amount” refers to an amount of the active ingredient capable of inducing an anticancer effect. Such an effective amount can be experimentally determined within the normal capabilities of those of ordinary skill in the art. [0140] As used herein, the term “treatment” may be used to mean both therapeutic treatment and preventive treatment. Prevention may be used to mean alleviating or reducing a pathological condition or disease in a subject. In one embodiment, the term “treatment” includes all applications or any form of medication for treating a disease in mammals, including humans. In addition, the above term includes inhibiting or slowing down a disease or the progression of a disease; restoring or repairing damaged or defective functions, thereby partially or completely alleviating a disease; or stimulating an inefficient process; or alleviating a serious disease.

[0141] As used herein, the term “efficacy” can be determined by one or more parameters, such as survival or disease-free survival over a period of time, such as 1 year, 5 years, or 10 years. In addition, the parameters can include suppression of the size of at least one tumor in a subject.

[0142] Pharmacokinetic parameters, such as bioavailability, and underlying parameters, such as clearance rate, can also affect efficacy. Therefore, “enhanced efficacy” (for example, improved efficacy) can be due to enhanced pharmacokinetic parameters and enhanced efficacy, and can be measured by comparing clearance rates and tumor growth in test animals or human subjects, or by comparing parameters such as survival, relapse rate, or disease-free survival.

[0143] Here, the term “therapeutically effective amount” or “pharmaceutically effective amount” refers to an amount of a compound or composition effective for preventing or treating a target disease, which is an amount that is sufficient to treat the disease with a reasonable benefit/risk ratio applicable to medical treatment and that does not cause side effects. The level of the effective amount may be determined according to factors including the health condition of the patient, the type and severity of the disease, the activity of the drug, the sensitivity to the drug, administration method, administration time, the route of administration and excretion rate, treatment period, the drug to be combined or concurrently used, and other factors well known in the medical field. In one embodiment, a therapeutically effective amount refers to an amount of a drug effective for treating cancer.

[0144] The pharmaceutical composition may be administered as an individual therapeutic agent or in combination with another therapeutic agent, may be administered sequentially or simultaneously with a conventional therapeutic agent, and may be administered in one dose or in multiple doses. It is important to administer an amount that can achieve the maximum effect with the minimum amount without side effects by considering all of the above factors, and this can be easily determined by those of ordinary skill in the art.

[0145] At this time, the pharmaceutical composition may further comprise a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier may be any carrier as long as it is non-toxic material suitable for delivery to a patient. Distilled water, alcohol, fats, waxes and inert solids may be included as a carrier. In addition, a pharmacologically acceptable adjuvant (buffering agent, dispersing agent) may be included in the pharmaceutical composition.

[0146] Specifically, the pharmaceutical composition may be prepared as a parenteral formulation according to the route of administration by a conventional method known in the art, including a pharmaceutically acceptable carrier in addition to the active ingredient. Here, “pharmaceutically acceptable” means that it does not inhibit the activity of the active ingredient and does not have toxicity beyond what the subject for application (prescription) can tolerate.

[0147] When the pharmaceutical composition is prepared as a parenteral formulation, it may be formulated in the form of an injection, a transdermal preparation, a nasal inhalant, and a suppository together with suitable carriers according to methods known in the art. When it is formulated as an injection, as a suitable carrier, sterile water, ethanol, polyol such as glycerol or propylene glycol, or a mixture thereof may be used, and Ringer's solution, PBS (Phosphate Buffered Saline) containing triethanolamine, or sterile water for injection, an isotonic solution such

as 5% dextrose, and the like may be preferably used. Methods for formulating pharmaceutical compositions are known in the art, and specific references can be made to literature [Remington's Pharmaceutical Sciences (19th ed., 1995)], etc. The above literature is considered a part of the present specification.

[0148] The preferred dosage of the pharmaceutical composition may be in the range of 0.01 ug/kg to 10 g/kg per day, or in the range of 0.01 mg/kg to 1 g/kg per day, depending on the patient's condition, body weight, sex, age, the severity of the patient, and the route of administration. The administration may be performed once a day or divided into several times per day. This dosage may be increased or decreased depending on the route of administration, the severity of the disease, gender, body weight, age, and the like, and therefore should not be construed as limiting the scope of the present invention in any way.

[0149] The subject to which the pharmaceutical composition can be applied (prescribed) is a mammal and a human, and particularly, the subject is preferably a human. In addition to the active ingredient, the pharmaceutical composition of the present invention may further comprise any compound or natural extract that has already been verified as safe and known to have anticancer activity in order to increase or enhance anticancer activity.

[0150] In another aspect of the present invention, there is provided a pharmaceutical composition for combination administration for treating or preventing cancer, comprising the extracellular vesicle and an immune anticancer agent as active ingredients.

[0151] Since the extracellular vesicles increase immune activity in the body, they can be used in combination with an immune checkpoint inhibitor, a conventionally-used anticancer agent.

[0152] The immune anticancer agent may be any one selected from the group consisting of an immune checkpoint inhibitor, a chimeric antigen receptor-T cell (CAR-T), a T cell receptor modified T cell (TCR-T), a tumor infiltrating lymphocyte (TIL), a bispecific T-cell engager (BiTE), a tumor vaccine, and an oncolytic virus, but is not limited thereto.

[0153] The immune checkpoint inhibitor may be any one selected from the group consisting of an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-B7-H4 antibody, an anti-HVEM antibody, an anti-TIM3 antibody, an anti-GAL9 antibody, an anti-LAG3 antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-BTLA antibody, and an anti-TIGIT antibody, but is not limited thereto. Specifically, it may be an anti-PD-1 antibody. In one embodiment, the immune checkpoint inhibitor may be Ipilimumab, Pembrolizumab, Nivolumab, Cemiplimab, Atezolizumab, Avelumab, Duralumab, or a combination thereof, but is not limited thereto.

[0154] As used herein, the term “immune checkpoint inhibitor” refers to a substance that inhibits the activity of an immune checkpoint protein that inhibits the differentiation, proliferation, and activity of immune cells, and is known to eliminate cancer cells by preventing cancer cells from evading the immune system.

[0155] In one embodiment, when comparing the administration of the extracellular vesicle alone with the administration of the extracellular vesicle in combination with an anti-PD-1 antibody, it was confirmed that the tumor growth inhibitory effect was significantly increased by combination administration.

#### Method for Preparing Novel Extracellular Vesicles

[0156] In another aspect of the present invention, there is provided a method for preparing extracellular vesicles, comprising: a) culturing human-derived cells in a serum-free cell culture medium supplemented with Poly I:C and monophosphoryl lipid A (MPLA); b) obtaining a culture solution during the culturing process; and c) filtering the culture solution obtained during the above process to remove particles larger than 200 nm and then isolating the extracellular vesicles.

[0157] In one embodiment, the serum-free cell culture medium may be additionally supplemented with zymosan. Specifically, the serum-free cell culture medium may be supplemented with Poly I:C and monophosphoryl lipid A (MPLA); or zymosan, Poly I:C, and monophosphoryl lipid A



(MPLA).

[0158] Overlapping contents are omitted in consideration of the complexity of the present specification, and terms not otherwise defined in the present specification have meanings commonly used in the art to which the present invention pertains.

[0159] Hereinafter, the present invention will be described in more detail through examples. These examples are intended only to illustrate the present invention, and it will be obvious to those of ordinary skill in the art that the scope of the present invention is not to be construed as being limited by these examples.

Preparation Example 1. Culturing of Wharton's Jelly-Derived Mesenchymal Stem Cells

[0160] The human Wharton's jelly-derived mesenchymal stem cells (WJ-MSC) were cultured in minimal essential media alpha modification ( $\alpha$ -MEM) (Welgene, South Korea) medium supplemented with 10% fetal bovine serum (Corning, USA) and 1% penicillin-streptomycin at 37° C. and 5% CO.sub.2 conditions. The human Wharton's jelly-derived mesenchymal stem cells were obtained from Samsung Medical Center with IRB approval and used under research consent.

Manufacturing Example 1. Isolation of Exosomes from Wharton's Jelly-Derived Mesenchymal Stem Cells Treated with Chemical Substance Cocktail

[0161] When the WJ-MSCs of Preparation Example 1 were passaged 7 to 13 times and the confluency of the WJ-MSCs reached 80% to 90%, the culture medium was removed and then washed with PBS (Welgene, South Korea). Thereafter, the cells were cultured in a serum-free  $\alpha$ -MEM medium supplemented with a chemical substance cocktail at 37° C. and 5% CO.sub.2 conditions for 24 hours to obtain a culture solution. The chemical substance cocktail consisted of 1  $\mu$ g/mL zymosan A (isolated from *Saccharomyces cerevisiae*) (Sigma-Aldrich, USA), 30 ng/mL polyinosinic-polycytidylic acid (Poly I:C) (Sigma-Aldrich, USA), and 1  $\mu$ g/mL monophosphoryl lipid A (MPLA) (InvivoGen, USA).

[0162] The obtained WJ-MSC culture solution was centrifuged at 4° C. and 3,000 $\times$ g conditions for 20 minutes to remove cells and large cell debris. The supernatant obtained through centrifugation was passed through a 0.2 m Minisart™ NML syringe filter (Sartorius, Germany) to remove particles larger than 200 nm. Thereafter, exosomes were isolated using a Labspinner (Live Cell Instrument, South Korea) equipment. The exosomes were obtained in a state existing in PBS and used directly for experiments or stored frozen at 80° C.

Comparative Example 1. Isolation of Exosomes from Wharton's Jelly-Derived Mesenchymal Stem Cells

[0163] When the WJ-MSCs of Preparation Example 1 were passaged 7 to 13 times and the confluency of the WJ-MSCs reached 80% to 90%, the culture medium was removed and then washed with PBS (Welgene, South Korea). Thereafter, the cells were cultured in a serum-free  $\alpha$ -MEM medium at 37° C. and 5% CO.sub.2 conditions for 24 hours to obtain a culture solution.

[0164] Exosomes were isolated from the obtained culture solution in the same manner as described in Manufacturing Example 1.

Comparative Example 2. Isolation of Exosomes from Wharton's Jelly-Derived Mesenchymal Stem Cells Treated with Poly I:C

[0165] When the WJ-MSCs of Preparation Example 1 were passaged 7 to 13 times and the confluency of the WJ-MSCs reached 80% to 90%, the culture medium was removed and then washed with PBS (Welgene, South Korea). Thereafter, the cells were cultured in a serum-free  $\alpha$ -MEM medium supplemented with 30 ng/mL of Poly I:C (Sigma-Aldrich, USA) at 37° C. and 5% CO.sub.2 conditions for 24 hours to obtain a culture solution.

[0166] Exosomes were isolated from the obtained culture solution in the same manner as described in Manufacturing Example 1.

Comparative Example 3. Isolation of Exosomes from HeLa Cells Treated with Chemical Substance Cocktail

[0167] Exosomes were isolated in the same manner as described in Manufacturing Example 1

using HeLa cells instead of WJ-MSCs. The HeLa cells were purchased from the ATCC and used.

**Manufacturing Example 2. Isolation in Bulk of Exosomes from Wharton's Jelly-Derived Mesenchymal Stem Cells Treated with Chemical Substance Cocktail**

[0168] When the WJ-MSCs of Preparation Example 1 were passaged 7 to 13 times and the confluency of the WJ-MSCs reached 80% to 90%, the culture medium was removed and then washed with PBS (Welgene, South Korea). Thereafter, the cells were cultured in a serum-free  $\alpha$ -MEM medium supplemented with a chemical substance cocktail at 37° C. and 5% CO<sub>2</sub> conditions for 24 hours to obtain a culture solution. The chemical substance cocktail consisted of 1  $\mu$ g/mL zymosan A (isolated from *Saccharomyces cerevisiae*) (Sigma-Aldrich, USA), 30 ng/mL polyinosinic-polycytidylic acid (Poly I:C) (Sigma-Aldrich, USA), and 1  $\mu$ g/mL monophosphoryl lipid A (MPLA) (InvivoGen, USA).

[0169] The obtained WJ-MSC culture solution was subjected to tangential flow filtration (TFF) to remove cells and large cell debris (0.65  $\mu$ m). The solution obtained by passing through the filter was concentrated in the same manner (TFF) using a MWCO 300,000 or 500,000 Da (Dalton) filter to remove impurities below the MWCO. Thereafter, buffer exchange (dialysis) with PBS, etc. was performed using the same method (TFF) to isolate exosomes.

**Comparative Example 4. Isolation in Bulk of Exosomes from Wharton's Jelly-Derived Mesenchymal Stem Cells**

[0170] When the WJ-MSCs of Preparation Example 1 were passaged 7 to 13 times and the confluency of the WJ-MSCs reached 80% to 90%, the culture medium was removed and then washed with PBS (Welgene, South Korea). Thereafter, the cells were cultured in a serum-free  $\alpha$ -MEM medium at 37° C. and 5% CO<sub>2</sub> conditions for 24 hours to obtain a culture solution.

[0171] Exosomes were isolated from the obtained culture solution in the same manner as described in Manufacturing Example 2.

**Comparative Example 5. Isolation in Bulk of Exosomes from iPSC-Derived Mesenchymal Stem Cells Treated with Chemical Substance Cocktail**

[0172] The induced pluripotent stem cells purchased from the ATCC were placed in a 12-well plate coated with vitronectin, and cultured for 5 days in  $\alpha$ -MEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS) by adding a chemical substance cocktail comprising SB4, SB431542, Y27632, PD0325901, SB202190, SP600125, Go6983, and CHIR99021. Cells differentiated into mesoderm were obtained, and then the cells isolated from the medium by treating with 0.25% Trypsin-EDTA were centrifuged at 300 RCF for 5 minutes.

Thereafter, a chemical substance cocktail comprising Y27632, PD0325901, SB202190, SP600125, Go6983 and CHIR99021 was added to  $\alpha$ -MEM medium supplemented with 10% FBS and 1% PS, and the collected cells were suspended, and then 1 $\times$ 10<sup>5</sup> cells per well were placed in a 6-well plate and cultured for 5 days to induce the differentiation into mesenchymal stem cells. The culture solution was obtained during the process of culturing the differentiated iPSC-derived mesenchymal stem cells (ciMSC) in a low-concentration DMEM medium supplemented with 10% FBS and 1% PS.

[0173] Exosomes were isolated from the obtained culture solution in the same manner as described in Manufacturing Example 2.

**Experimental Example 1. Analysis of Characteristics of Exosomes Isolated from Wharton's Jelly-Derived Mesenchymal Stem Cells Treated with Chemical Substance Cocktail**

**Experimental Example 1.1. Measurement of Exosome Size**

[0174] The size and size distribution of exosomes isolated from human Wharton's jelly-derived mesenchymal stem cells in Comparative Example 1 (CTRL Exo) and exosomes isolated from human Wharton's jelly-derived mesenchymal stem cells treated with a chemical substance cocktail in Manufacturing Example 1 (CHEM Exo) were measured using nanoparticle tracking analysis (NTA; NS300, Malvern Panalytical, UK), and the results are shown in FIG. 1.

[0175] As shown in FIG. 1, it was confirmed that the sizes of CTRL Exo and CHEM Exo were

distributed between 50 and 200 nm.

#### Experimental Example 1.2. Confirmation of Marker Expression of Exosomes

[0176] In order to confirm whether exosomes isolated from human Wharton's jelly-derived mesenchymal stem cells in Comparative Example 1 (CTRL Exo) and exosomes isolated from human Wharton's jelly-derived mesenchymal stem cells treated with a chemical substance cocktail in Manufacturing Example 1 (CHEM Exo) express exosome-specific markers, Western blot was performed. Specifically, as primary antibodies, the exosome-positive markers, CD63 monoclonal antibodies (mAbs; Abcam, UK), CD81 mAbs (Bioss Antibodies, USA), CD9 mAbs (Cell Signaling Technology, USA) and TSG101 mAbs (Abcam, UK), and the exosome-negative markers, GM130 polyclonal antibodies (pAbs; Bioss Antibodies, USA) and Calnexin mAbs (Bioss Antibodies, USA), were used. For detection of primary antibodies, goat anti-mouse or goat anti-rabbit IgG H&L (Abcam, UK) conjugated with horseradish peroxidase (HRP) was used as a secondary antibody. The results of Western blot are shown in FIG. 2.

[0177] As shown in FIG. 2, it was confirmed that both CTRL Exo and CHEM Exo expressed CD9, CD63, CD81 proteins belonging to the Tetraspanin family, which are membrane proteins of exosomes, and TSG101, a multivesicular body protein. In addition, it was confirmed that calnexin, a membrane-resident protein present in the endoplasmic reticulum, and GM130, a Golgi marker, were not expressed. This confirmed that the exosomes isolated in Manufacturing Example 1 were pure exosomes that did not comprise cellular organelles other than exosomes.

#### Experimental Example 2. Analysis of Cytokines Present in Exosomes

[0178] A cytokine ELISA was performed to analyze cytokines present in exosomes. Cytokine analysis was performed through Creative Biolabs' Exosomal cytokine profiling service, which is an analysis using the Bio-Plex Pro human cytokine screening 48-plex panel (Bio-Rad, #1200283) that can check the expression of multiple cytokines at once. Data analysis was performed from August to September 2021.

[0179] Specifically, S1 to S8 are samples for creating a standard curve, and the concentrations were set to decrease in order from S1, and PBS was used as a negative control. CTRL Exo is an exosome isolated from human Wharton's jelly-derived mesenchymal stem cells in Comparative Example 1, CHEM Exo is an exosome isolated from human Wharton's jelly-derived mesenchymal stem cells treated with a chemical substance cocktail in Manufacturing Example 1, POLYI:C Exo is an exosome isolated from human Wharton's jelly-derived mesenchymal stem cells treated with Poly I:C in Comparative Example 2, and HeLa CHEM Exo is an exosome isolated from HeLa cells treated with a chemical substance cocktail in Comparative Example 3.

[0180] In order to confirm whether the analysis results of cytokines present in the exosomes isolated from human Wharton's jelly-derived mesenchymal stem cells in Manufacturing Example 1 are specific to mesenchymal stem cells, HeLa cells, the most commonly used cell line, were treated with the chemical substance cocktail described in Manufacturing Example 1, and then exosomes were isolated to confirm the expression of cytokines in Comparative Example 3, and the results are shown in FIG. 3.

[0181] As shown in FIG. 3, it was confirmed that the expression of IL-6, IL-8, MCP-3, MCP-1, IP-10, and RANTES was strongly increased in the exosomes isolated from human Wharton's jelly-derived mesenchymal stem cells treated with a chemical substance cocktail (CHEM Exo) compared to the exosomes that were not treated with the chemical substance cocktail (CTRL Exo) and the exosomes that were treated with only Poly I:C (POLYI:C Exo). In addition, it was confirmed that when treated with the chemical substance cocktail having the same composition, the expression of IL-6, IL-8, MCP-3, MCP-1, IP-10, and RANTES was strongly increased in the exosome isolated from human Wharton's jelly-derived mesenchymal stem cells (CHEM Exo), whereas the expression of the corresponding cytokines was not increased in the exosomes isolated from HeLa cells (HeLa CHEM Exo).

#### Experimental Example 3. Analysis of miRNAs Present in Exosomes Isolated from Wharton's Jelly-

# Derived Mesenchymal Stem Cells Treated with Chemical Substance Cocktail

[0182] In order to analyze miRNAs present in exosomes, the Extracellular vesicle characterization service of Creative Biolabs was used. Specifically, miRNAs contained in exosome samples were extracted, the library was constructed, and then sequencing was performed. By comparing the resulting sequence information with the existing miRbase database, miRNAs that were relatively more expressed in each exosome sample were analyzed, and the results are shown in FIG. 4. Data analysis was performed from August to September 2021.

[0183] Comparative Example 1 is an exosome isolated from human Wharton's jelly-derived mesenchymal stem cells (CTRL Exo), Manufacturing Example 1 is an exosome isolated from human Wharton's jelly-derived mesenchymal stem cells treated with a chemical substance cocktail (CHEM Exo), Comparative Example 2 is an exosome isolated from human Wharton's jelly-derived mesenchymal stem cells treated with Poly I:C (POLY IC Exo), and Comparative Example 3 is an exosome isolated from HeLa cells treated with a chemical substance cocktail (HeLa CHEM Exo). The results of measuring the types of miRNAs present in each exosome and their expression intensities are shown in Table 1 below.

TABLE-US-00001 TABLE 1 Compar- Compar- Compar- Manufacturing active active active Example									
1 Example 2 Example 1 Example 3 Novel	miRNA-67	792	292	0	0	hsa-miR-199a-3p	322	383	54
NovelmiRNA-298	199	0	0	0	hsa-miR-145-5p	106	80	5	2
NovelmiRNA-109	88	0	0	0	hsa-miR-432-5p	81	27	0	0
NovelmiRNA-88	66	0	0	0	NovelmiRNA-230	68	0	0	0
hsa-miR-200b-3p	63	47	0	0	NovelmiRNA-169	56	0	0	0
hsa-miR-487b-3p	39	35	0	0	hsa-miR-200a-3p	41	21	0	0
hsa-miR-214-3p	36	53	3	0	NovelmiRNA-208	35	0	0	0
NovelmiRNA-164	34	0	0	0	NovelmiRNA-225	32	0	0	0
NovelmiRNA-174	30	0	0	0	hsa-miR-708-5p	22	0	2	0
hsa-miR-124-3p	21	0	2	1	NovelmiRNA-150	19	0	0	0
hsa-miR-135a-5p	17	27	0	0	NovelmiRNA-64	16	0	0	0
NovelmiRNA-92	16	0	0	0	NovelmiRNA-168	16	0	0	0
NovelmiRNA-22	14	0	0	0	NovelmiRNA-35	13	0	0	0
NovelmiRNA-161	12	0	0	0	hsa-miR-3940-3p	11	0	0	0
hsa-miR-219a-3p	11	0	0	0	hsa-miR-212-3p	11	0	0	5
NovelmiRNA-76	10	0	0	0	hsa-miR-323a-3p	9	6	0	1
hsa-miR-654-3p	7	8	0	0	hsa-miR-204-5p	7	1	0	1
hsa-miR-136-3p	7	1	0	0	NovelmiRNA-39	7	0	0	0
NovelmiRNA-175	7	0	0	0	hsa-miR-134-5p	6	23	2	0
hsa-miR-454-3p	5	0	1	0	NovelmiRNA-286	5	3	0	0
hsa-miR-542-3p	4	0	0	0	hsa-miR-433-3p	4	0	0	0
hsa-miR-376a-2-3p	1	35	0	0	hsa-miR-376a-3p	1	35	0	0
hsa-miR-664a-5p	1	8	0	0	hsa-miR-150-3p	0	4	0	0
hsa-miR-3909	0	5	0	0	hsa-miR-618	0	6	0	0
NovelmiRNA-52	0	29	0	0	NovelmiRNA-172	0	550	0	0
NovelmiRNA-218	0	42	0	0	NovelmiRNA-220	0	11	0	0
NovelmiRNA-222	0	53	0	0	NovelmiRNA-266	0	19	0	0
NovelmiRNA-283	0	532	0	0					

[0184] As shown in Table 1, it was confirmed that the expression intensities of NovelmiRNA-67, hsa-miR-199a-3p, NovelmiRNA-298, hsa-miR-145-5p, NovelmiRNA-281, NovelmiRNA-109, hsa-miR-432-5p, NovelmiRNA-230, NovelmiRNA-88, hsa-miR-200b-3p, NovelmiRNA-169, hsa-miR-200a-3p, hsa-miR-487b-3p, hsa-miR-214-3p, NovelmiRNA-208, NovelmiRNA-164, NovelmiRNA-225, NovelmiRNA-174, hsa-miR-708-5p, hsa-miR-124-3p, NovelmiRNA-150, hsa-miR-135a-5p, NovelmiRNA-64, NovelmiRNA-92, NovelmiRNA-168, NovelmiRNA-22, NovelmiRNA-35, NovelmiRNA-161, hsa-miR-3940-3p, hsa-miR-219a-3p, hsa-miR-212-3p, NovelmiRNA-76, hsa-miR-323a-3p, hsa-miR-654-3p, hsa-miR-204-5p, hsa-miR-136-3p, NovelmiRNA-39, NovelmiRNA-175, hsa-miR-134-5p, hsa-miR-454-3p, NovelmiRNA-286, hsa-miR-542-3p, and hsa-miR-433-3p were specifically increased in the exosomes isolated in Manufacturing Example 1 (CHEM Exo) compared to the exosomes isolated in Comparative Examples i to 3.

[0185] Specifically, it was confirmed that the expression intensities of hsa-miR-199a-3p and hsa-miR-200b-3p, known to inhibit cell proliferation and induce apoptosis, hsa-miR-145-5p, hsa-miR-432-5p and hsa-miR-200a-3p, known to inhibit cell migration and invasion, hsa-miR-214-3p and hsa-miR-708-5p, known to inhibit cell proliferation and cell cycle progression, hsa-miR-487b-3p, known to inhibit chemoresistance and metastasis of osteosarcoma, hsa-miR-124-3p, known to

function as a tumor suppressor, and hsa-miR-135a-5p, known to inhibit tumor invasion, were specifically increased.

[0186] Meanwhile, it was confirmed that the expression intensities of hsa-miR-376a-2-3p, hsa-miR-376a-3p, hsa-miR-664a-5p, hsa-miR-150-3p, hsa-miR-3909, hsa-miR-618, NovelmiRNA-52, NovelmiRNA-172, NovelmiRNA-218, NovelmiRNA-220, NovelmiRNA-222, NovelmiRNA-266, and NovelmiRNA-283 were relatively decreased in the exosomes isolated from human Wharton's jelly-derived mesenchymal stem cells treated with zymosan, Poly I:C, and monophosphoryl lipid A (MPLA) in Manufacturing Example 1 (CHEM Exo) compared to the exosomes isolated from human Wharton's jelly-derived mesenchymal stem cells treated with Poly I:C in Comparative Example 2 (POLY IC Exo).

Experimental Example 4. Analysis of mRNAs Present in Exosomes Isolated from Wharton's Jelly-Derived Mesenchymal Stem Cells Treated with Chemical Substance Cocktail

[0187] In order to compare the mRNA expression patterns of exosomes isolated from human Wharton's jelly-derived mesenchymal stem cells treated with a chemical substance cocktail in Manufacturing Example 1 and exosomes isolated from human Wharton's jelly-derived mesenchymal stem cells in Comparative Example 1, a microarray experiment was performed. Specifically, using the Extracellular vesicle characterization service of Creative Biolabs, mRNAs contained in each exosome sample were extracted, and mRNAs that were relatively more expressed were analyzed through microarray. Data analysis was performed from August to September 2021. As a result of performing microarray, it was confirmed that the expression levels of a total of 275 types of mRNA, including MED13L, were significantly increased in human Wharton's jelly-derived mesenchymal stem cells treated with a chemical substance cocktail. Table 2 below lists 275 types of mRNA.

TABLE-US-00002 TABLE 2 MED13L lnc-EXT1-2 LOC100507420 lnc-PHYHD1-1 SHARPIN lnc-TOMM20L-1 LIN54 OPA3 GIGYF2 ASMTL-AS1 lnc-CDK5R1-1 C17orf74 XLOC\_I2\_014245 RNF151 HMOX1 ETV3 SLC12A2 lnc-NR5A1-1 BAGE SHARPIN SLC35G1 TTC28 DMTN ADRA2C LOC101928207 SMARCA4 ADRA2A SMPD4 lnc-SLC43A1-1 TRAI ACAT2 NLGN2 TXLNB ENTPD8 lnc-ANTXRL-2 ADAM12 lnc-FBXO25-4 SPRR1A NYAP1 LINC01135 ARFGEF1 LOC100506603 DISP1 lnc-GLIPR1L1-1 BZRAP1 CELF3 LOC399886 LOC100130285 CCNT2-AS1 LOC101926983 DNAJB5 RCC2 lnc-SLC17A9-1 NAV1 WDR6 lnc-WRNIP1-2 KIAA0040 lnc-SELV.1-1 TBX21 C19orf81 ACTL8 HOXB9 lnc-ROM1-2 GNAZ NAMA lnc-TM2D3-2 JMJD8 lnc-RABEPK-1 DNM3OS LIM2 GCN1L1 LINC01146 lnc-RRP8-1 PRKAG2-AS1 FAM155A-IT1 LOC101929337 lnc-GIT1-1 lnc-RP11- lnc-MFSD9-4 lnc-RPP30-2 234B24.6.1-1 FAM217A VRTN lnc-TMEM189- lnc-RPGRIP1-2 ZNF264 UBE2V1-2 XLOC\_I2\_013467 NHP2 LAMTOR2 ABCC6 MALAT1 SNHG4 KIF26A BTK LRFN3 HTT-AS NUDT13 HAUS5 lnc-BSND-1 lnc-COL1A1-2 ZDHHC22 MCTS1 lnc-SOD2-2 C2orf71 lnc-PPAPDC3-2 SEPHS2 ATG4D APOL3 lnc-C17orf63-2 CTU1 ABTB1 NPEPL1 lnc-POLR2L-1 lnc-CHGA-1 lnc-PABPC4-1 lnc-RP11- 791J7.2.1-1 YEATS2 lnc-SUPT3H-1 ABCD1 LOC100507165 CCDC28B SPEN lnc-XRN2-3 NCAN LOC102724861 LOC284263 GGT6 RTP2 C9orf106 SPTSSB lnc-PPIC-2 lnc-RP11- TERT AGAP2 lnc-C20orf96-4 CDC42BPB 770J1.4.1-1 MIAT TNFRSF12A EMC6 DCDC5 C9orf173-AS1 lnc-HMCN1-2 LOC645427 CD86 lnc-CRYL1-1 C9orf139 RETN lnc-CTR9-5 LOC100129175 LOC100506114 FGD5 SP6 SENP7 SLC48A1 CCNT2-AS1 lnc-RP11- 322L20.1.1-7 RCOR2 lnc-C17orf97-4 RPL28 lnc-SCAMP5-1 RAD51 lnc-AC016251.1-2 ARNTL BGN BRD1 MEIOB lnc-PRKAA2-1 lnc-RTL1-1 BZW1 lnc-ELAVL4-3 lnc- ADAMTS14-1 RANBP3 ZNF713 lnc-C6orf221-2 CHRDL1 PCDHA13 lnc-SH2B3-1 XLOC\_I2\_005871 SLC20A2 lnc-FUT8-1 XLOC\_I2\_011901 QSOX1 HYALP1 lnc-DNAJC7-1 CACNA1H SP2-AS1 SNAR-G1 LINC01264 LINC01314 ARSA SEC22A LMO2 lnc-VAPB-1 PHF7 TIMM8B LINC01197 ARHGAP23 GS1-24F4.2 ATAD1 LOC101927533 ATG9A LRIT1 FNDC7 REP15 GM2A PLLP B3GNT8 C1orf226 XLOC\_I2\_013547 SLC4A11 STARD7-AS1 lnc-STEAP1B-1 lnc-SLC25A26-1 C11orf95 TMEM100 SNORD3B-1 TMEM240 FAM110B LARP6

lnc-TMEM135-2 CLSTN3 IER5 HELZ2 lnc-FAM160A1-1 ATG16L2 LINC01508 TMX2  
 GOLGA2P5 MMP11 lnc-GTPBP8-1 lnc-EPHB6-1 TMEM132C TSSK3 LOC400958 PSMC2  
 GPX3 lnc-SOX6-1 WFDC21P lnc-LINC00273-1 lnc-DCAF4L2-2 EDEM3 lnc-CPXM2-2  
 TNFRSF25 XLOC\_12\_014217 LOC101927210 P2RX6 BCL3 PPT1 C14orf169 SLC22A7  
 LOC101929450 RPA4 EBLN2 lnc-PPP2R2C-1 LOC102724416 KCTD5 lnc-XRN1-1 lnc-GATA5-  
 2 FLII lnc-UQCRFS1-9 lnc-FTSJD2-1 MARVELD1 WAC MTUS2-AS1 MDFI lnc-TRIM41-3  
 PPP6R1 CHKA LOC102546226 PRSS8 LOC100507377 TBC1D31 SLC5A10 SEMA6C  
 LRRFIP2 SNORD38A

Experimental Example 5. Confirmation of In Vitro Effect of Exosomes Isolated from Wharton's Jelly-Derived Mesenchymal Stem Cells Treated with Chemical Substance Cocktail

Experimental Example 5.1. Culturing of Mouse Macrophage Cell Line and Exosome Treatment [0188] RAW 264.7 (ATCC, TTB-71), a mouse macrophage cell line, was cultured in Dulbecco's modified Eagle medium with high glucose (DMEM; Welgene, South Korea) medium supplemented with 1000 heat-treated fetal bovine serum (Corning, USA) and 1% penicillin-streptomycin at 37° C. and 500 CO.sub.2 conditions.

[0189] RAW 264.7 cells were seeded at 1×10<sup>5</sup> cells per well in a 48-well plate (Corning, USA) and cultured for 24 hours in serum free DMEM medium. The medium was supplemented with either no treatment (CTRL), 1×10<sup>8</sup> exosomes isolated from human Wharton's jelly-derived mesenchymal stem cells in Comparative Example 1 (CTRL Exo), 1×10<sup>8</sup> exosomes isolated from human Wharton's jelly-derived mesenchymal stem cells treated with a chemical substance cocktail in Manufacturing Example 1 (CHEM Exo), or 10 ng/mL of lipopolysaccharide (Sigma-Aldrich, USA) (LPS).

Experimental Example 5.2. Analysis of Amount of Nitric Oxide Production

[0190] In order to analyze the amount of nitric oxide (NO) production of RAW 264.7 following CTRL, CTRL Exo, CHEM Exo or LPS treatment, the culture media obtained from cells treated with CTRL, CTRL Exo, CHEM Exo, and LPS, respectively, were transferred to new 96-well plates (VWR, USA), and then the NO concentration was measured using a Nitric Oxide plus detection kit (iNtRON Biotech, South Korea), and the results are shown in FIG. 5.

[0191] As shown in FIG. 5, it was confirmed that the amount of nitric oxide production was significantly increased when treated with CHEM Exo compared to when an inflammatory response was induced by treatment with LPS. This confirmed that the exosomes isolated from human Wharton's jelly-derived mesenchymal stem cells treated with a chemical substance cocktail in Manufacturing Example 1 induce macrophage activation through the secretion of nitric oxide, thereby enhancing the inflammatory response.

Experimental Example 5.3. Measurement of Expression Level of Inflammation-Related mRNA

[0192] In order to analyze the expression levels of cytokines and chemokines in RAW 264.7 following the treatment with CTRL, CTRL Exo, CHEM Exo, or LPS, real-time polymerase chain reaction (quantitative reverse transcription PCR, RT-qPCR) was performed. Specifically, the cells treated with CTRL, CTRL Exo, CHEM Exo, and LPS, respectively, were lysed, and then RNAs were extracted and purified using TaKaRa MiniBEST universal RNA extraction kit (Takara Bio Inc., Japan). Thereafter, complementary DNAs (cDNA) of the RNAs were synthesized using the PrimeScript 1st strand cDNA synthesis kit (Takara Bio Inc., Japan). The expression levels of mouse interleukin-6 (IL-6), IL-1β, tumor necrosis factor-α (TNF-α), nitric oxide synthase 2 (NOS2), and cyclooxygenase 2 (COX2) were measured by performing RT-qPCR with the synthesized cDNAs, and the results are shown in FIG. 6. GAPDH was used as a reference gene for normalization of gene expression levels, and the sequence information of the primers used for RT-qPCR is as shown in Table 3 below.

TABLE-US-00003	TABLE	3	SEQ	Annealing	ID	Temperature	Size	Gene	Primer	NO (° C.)	(bp)
GAPDH	Forward	CAT	CAC	TGC	CAC	CCA	GAA	GAC	TG	1	63.3
	Reverse	ATG	CCA	GTG	AGC	TTC	CCG	AG	2	65.2	
IL-6	Forward	AGT	TGC	CTT							

CTT GGG ACT GAT 3 59.3 159 Reverse TCC ACG ATT TCC CAG AGA ACA  
 4 59 IL-12 Forward AGG TCA CAC TGG ACC AAA GG 5 60.5 173 Reverse TGG  
 TTT GAT GAT GTC CCT GA 6 56.4 IL-1 $\beta$  Forward TTC CTG TTG TCT  
 ACA CCA ATG C 7 60.3 162 Reverse CGG GCT TTA AGT GAG TAG GAG  
 A 8 62.1 TNF- $\alpha$  Forward ACG GCA TGG ATC TCA AAG AC 9 60.1 116 Reverse  
 GTG GGT GAG GAG CAC GTA GT 10 60.2 NOS2 Forward GAA CTG TAG  
 CAC AGC ACA GGA AAT 11 61.6 158 Reverse CGT ACC GGA TGA GCT  
 GTG AAT 12 60.2 COX2 Forward CAG TTT ATG TTG TCT GTC CAG AGT  
 TTC 13 60.5 127 Reverse CCA GCA CTT CAC CCA TCA GTT 14 60.6

[0193] As shown in FIG. 6, it was confirmed that the expression intensities of the inflammatory cytokines IL-6, IL-12, TNF- $\alpha$ , and IL-1 $\beta$  and the inflammation-related enzymes NOS2 and COX2 were significantly increased when treated with CHEM exosomes compared to when treated with CTRL or CTRL Exo. In addition, it was confirmed that the expression of IL-6, IL-12, IL-1 $\beta$ , NOS2, and COX2 was significantly increased compared to treatment with LPS, which is generally known to induce an inflammatory response, indicating that the intensity of the immune response induced by CHEM Exo is significant. All of the increased cytokines are pro-inflammatory cytokines, which activate dendritic cells, macrophages, T cells, and B cells. Therefore, these experimental results suggest that CHEM Exo of Manufacturing Example 1 can induce immune cell activation. In addition, the results showing increased secretion of proinflammatory cytokines compared to LPS suggest that the immune activation effect of CHEM Exo of Manufacturing Example 1 is very excellent.

Experimental Example 6. Confirmation of In Vivo Effect of Exosomes Isolated in Bulk from Wharton's Jelly-Derived Mesenchymal Stem Cells Treated with Chemical Substance Cocktail

Experimental Example 6.1. Preparation of Mouse Colorectal Cancer Cancer Cell Line

[0194] The mouse colorectal cancer cancer cell line CT26 (ATCC, CRL-2638) was suspended in RPMI1640 medium supplemented with 10% heat-treated fetal bovine serum (Gibco, 10082-147) and 1% penicillin-streptomycin, placed in a cell culture flask, and cultured in an incubator at 37° C. and 5% CO<sub>2</sub> conditions. After the culture was completed, the cells were washed with PBS, and then 10-fold diluted 2.5% Trypsin-EDTA (Gibco, 15090) was added to detach the cells. Thereafter, centrifugation was performed at 1,000 rpm for 5 minutes, the supernatant was removed, and the resulting cell suspension was transferred to a fresh medium. The cell viability was confirmed using a microscope, and then the cell line was prepared by diluting it in DPBS to a concentration of 5.0 $\times$ 10<sup>6</sup> cells/mL.

Experimental Example 6.2. Preparation of Mouse Colorectal Cancer Cancer Model

[0195] BALB/c mice were acclimatized to the breeding environment for 7 days, and then the day before transplantation of the CT26 cell line, the dorsal skin of the mice was shaved using an electric razor. When transplanting the cell line, the injection site was disinfected with 70% alcohol, the skin on the right dorsal side of the mouse was pulled with the thumb and index finger to create a space between the skin and muscle, and then the cells were administered subcutaneously using an insulin syringe into the subcutaneous space between the thumb and index finger from the anterior aspect of the animal at a dose of 5.0 $\times$ 10<sup>5</sup> cells/0.1 mL/head. After cell line inoculation, when the tumor size at the transplantation site reached about 300 mm<sup>3</sup>, the mice were divided into seven groups of six mice per group so that the tumor sizes in each group were evenly distributed.

Experimental Example 6.3. Administration of Exosomes to a Mouse Colorectal Cancer Cancer Model

[0196] The seven groups divided above were administered with PBS, exosomes isolated in bulk from human Wharton's jelly-derived mesenchymal stem cells in Comparative Example 4 (Ctrl Exo), anti-PD-1 antibody (BioXCell, #BP0273), exosomes isolated in bulk from human Wharton's jelly-derived mesenchymal stem cells treated with a chemical substance cocktail in Manufacturing Example 2 (IEP-01), exosomes isolated from iPSC-derived mesenchymal stem cells treated with a

chemical substance cocktail in Comparative Example 5 (IEP-01-i), a combination of IEP-01 and an anti-PD-1 antibody, and a combination of IEP-01-i and an anti-PD-1 antibody, respectively. The substances were administered three times a week for a total of six times over two weeks. CTRL Exo, IEP-01, and IEP-01-i were administered intravenously at a dose of  $5 \times 10^8$  particles/head, and the anti-PD-1 antibody was administered intraperitoneally at a dose of 5 mg/kg.

#### Experimental Example 6.4. Measurement of Body Weight and Tumor Volume of Mouse Colorectal Cancer Model

[0197] The body weight and tumor volume were measured three times a week from the start date of administration of the above substances. The tumor volume was calculated by measuring the long axis and short axis of the tumor using a caliper and calculating the tumor volume using the following equation, and the results are shown in FIGS. 7 and 8. In addition, the body weight of each mouse was measured, and the results are shown in FIG. 9.

[00001]  $\text{Tumor volume (mm}^3\text{)} = ab^2 / 2$  ( $a$ : long axis length,  $b$ : short axis length) [Equation 1]

[0198] As shown in FIGS. 7 and 8, when administered alone, the tumor growth inhibitory effect was the most excellent in the group administered with exosomes isolated in bulk from human Wharton's jelly-derived mesenchymal stem cells treated with a chemical substance cocktail in Manufacturing Example 2 (IEP-01), and it was confirmed that the tumor growth inhibitory effect was increased when the exosomes isolated from mesenchymal stem cells treated with a chemical substance cocktail (IEP-01 or IEP-01-i) were administered in combination with the anti-PD-1 antibody rather than when administered alone. Meanwhile, as shown in FIG. 9, it was confirmed that there was almost no change in the body weight of the mice depending on each administered substance.

#### Experimental Example 6.5. Measurement of Tumor Weight of Mouse Colorectal Cancer Model

[0199] Fifteen days after the start date of administration of the above substances, all surviving mice were anesthetized, then blood was collected from the posterior vena cava using a syringe, the abdominal aorta and posterior vena cava were cut to exsanguinate and kill the mice, and then the tumors were extracted. The extracted tumors were lightly washed with PBS, dried, and photographed. The results are shown in FIG. 11. The weights were measured, and the results are shown in FIG. 10.

[0200] As shown in FIGS. 10 and 11, the tumor weight of the group administered with exosomes isolated in bulk from human Wharton's jelly-derived mesenchymal stem cells treated with a chemical substance cocktail in Manufacturing Example 2 (IEP-01) was the lowest, and it was confirmed that the tumor reduction effect was increased when the exosomes isolated from iPSC-derived mesenchymal stem cells treated with a chemical substance cocktail in Comparative Example 5 (IEP-01-i) were administered in combination with the anti-PD-1 antibody rather than when administered alone.

#### Experimental Example 6.6. Functional Comparison of Exosomes Isolated from Human Wharton's Jelly-Derived Mesenchymal Stem Cells Treated with Poly I:C Alone and Treated with Chemical Substance Cocktail

[0201] In order to confirm whether the proinflammatory cytokine secretion effect of exosomes isolated in bulk from human Wharton's jelly-derived mesenchymal stem cells treated with a chemical substance cocktail in Manufacturing Example 2 (IEP-01) results from the exosomes themselves or the chemical substance cocktail used in Manufacturing Example 2, the following in vitro experiment was performed.

[0202] Specifically, the RAW 264.7 cell line was treated with exosomes isolated in bulk in Manufacturing Example 2 (IEP-01) or with Poly I:C alone, and then RT-PCR was performed in the same manner as described in Experimental Example 5.3. to measure the expression level of IL-6, a proinflammatory cytokine, and the results are shown in FIG. 12.



[0203] As shown in FIG. 12, when the concentration of Poly I:C was calculated based on the number of the treated IEP-01, it was confirmed that the expression of IL-6 was increased at a low concentration when treated with IEP-01 compared to when treated with Poly I:C alone. Specifically, it was confirmed that when the expression level of IL-6 mRNA was 4,000, 35 µg of Poly I:C was required, but in the case of IEP-01, 13.8 µg of Poly I:C was used. These results demonstrate that IEP-01 can achieve the same effect with Poly I:C at a concentration 2.5 times lower than when Poly I:C is used alone. In other words, it was confirmed that the proinflammatory cytokine secretion of IEP-01 was not the effect of Poly I:C alone, but rather the result of the combined action of cytokines, miRNA, and mRNA present in IEP-01.

#### Experimental Example 7. Comparative Experiment of Exosomes by Cell Type Obtained

##### Experimental Example 7.1. Isolation of Exosomes from Cells Treated with a Chemical Substance Cocktail

[0204] The WJ-MSC-derived exosomes, the exosomes derived from ciMSC differentiated from the iPSCs, and the exosomes obtained from the HEK293T cell line were compared by the treated chemical substance.

[0205] One day before obtaining the cell culture solution, the culture solution of the cells being grown was removed, washed with PBS, and the culture medium containing no FBS was used as the naive exosome group. In addition, exosomes obtained from the 3-chem group treated with poly I:C 30 µg/ml, MPLA 1 µg/ml, and Zymosan 1 µg/ml were designated as IEP-01. In addition, exosomes obtained from the 2-chem group treated with poly I:C 30 µg/ml and MPLA 1 µg/ml were designated as IEP-01'. Exosomes obtained from ciMSCs were additionally indicated with "i", and exosomes obtained from HEK293T were additionally indicated with "h". The cell culture solution was collected from each experimental group after culturing for 24 hours.

[0206] The exosome samples from the cell culture solution were isolated using tangential flow filtration. In order to label the exosomes, the exosome samples were cultured with PKH67 lipophilic fluorescent dye for 5 minutes at room temperature, and then washed twice with PBS containing a sufficient amount of 10% BSA. The labeled exosomes were concentrated using an Amicon 100 kDa MWCO filter column and quantified by NTA.

##### Experimental Example 7.2. Analysis of mRNAs Present in Each Exosome

[0207] Raw264.7 cells, a mouse-derived macrophage cell line, were treated with the exosome 1000 ptc/cell that had been obtained from each cell group treated with compounds, or 1 ng/mL of LPS for 24 hours. Thereafter, mRNA was isolated from Raw264.7 cells, and then cDNA was synthesized through reverse transcription. qRT-PCR was performed using TaqMan probes to confirm the expression of IL-6, iNOS2, and IL-1β using the synthesized cDNA. LPS was used as a positive control group to activate Raw264.7. The expression of each gene was quantified as a relative value based on GAPDH.

[0208] As shown in FIGS. 13a to 13c, it was confirmed that the expression of IL-6, IL-1β, or iNOS2 was not induced in Raw264.7 cells from the group treated with naive exosomes among the WJ-MSC-derived exosomes, whereas the expression of IL-6, IL-1β, or iNOS2 was induced more strongly in the exosomes obtained from the groups treated with 2-chem and 3-chem than that induced by LPS.

[0209] In addition, as shown in FIGS. 13d and 13e, similar to the exosomes obtained from WJ-MSCs, it was confirmed that the expression of IL-6 or iNOS was not induced in the group treated with naive exosomes among the ciMSC-derived exosomes, whereas the expression of IL-6 or iNOS2 was induced more strongly in the exosomes obtained from the group treated with 2-chem and 3-chem than that induced by LPS.

[0210] In addition, as shown in FIGS. 13f and 13g, similar to the exosomes obtained from WJ-MSCs and ciMSCs, the expression of IL-6 and IL-1β was not induced in the group treated with naive exosomes among the exosomes derived from HEK293T cell line, whereas a significant increase in the expression of IL-6 or IL-1β was observed in the exosomes obtained from the groups

treated with 2-chem and 3-chem.

[0211] These results suggest that treatment of various cell lines with polyLC and MPLA can secrete exosomes capable of activating Raw264.7 macrophages.

#### Experimental Example 8. Confirmation of Degree of Uptake of ciMSC-Derived Exosomes by Cell Types

[0212] The degree of uptake of ciMSC-derived exosomes was compared by PBMC and immune cell types.

[0213] For exosome uptake experiment, human peripheral blood mononuclear cells (CTLs) were cultured with labeled ciMSC-derived exosomes or unlabeled ciMSC-derived exosomes prepared above at a concentration of 3000 particles/cell in a CO.sub.2 incubator at 37° C. for 4 hours and washed twice with PBS. For immunotyping, the cells were cultured with fluorescence-labeled antibodies at 4° C. for 3 minutes, then washed with PBS containing 2% FBS, and analyzed using a Cytoflex flow cytometer. All data were analyzed using Kaluza software.

[0214] At this time, for immunotyping, APC anti-human CD3 (Biolegend, 300312), APC anti-human CD19 (Biolegend, 302212), Brilliant Violet 421™ anti-human CD19 (Biolegend, 302233), Brilliant Violet 421™ anti-human CD56 (NCAM) (Biolegend, 362551), Brilliant Violet 421™ anti-human CD11b (Biolegend, 301323), Brilliant Violet 421™ anti-human CD11c (Biolegend, 301627), PerCP/Cyanine5.5 anti-human CD14 (Biolegend, 367110), PE anti-human HLA-DR, DP, DQ (MHC class II) (Biolegend, 361716) antibodies were used.

[0215] As shown in FIGS. 14a to 14c, it was confirmed that the uptake of ciMSC-derived exosomes labeled with PKH fluorescence was significantly increased, especially in dendritic cells, monocytes, and macrophages among PBMCs.

#### Experimental Example 9. Confirmation of Macrophage Activation and Differentiation by ciMSC-Derived Exosomes

##### Experimental Example 9.1. Confirmation of Macrophage Activation by Exosomes

[0216] In order to confirm whether ciMSC-derived exosomes activate macrophages, Raw264.7 cells, a mouse-derived macrophage cell line, were treated with 10 ng/ml of LPS or 1000 ptc/ml of IEP-01i' (2-chem-treated ciMSC-derived exosomes) for 24 hours. After treatment, the cells were washed with PBS and cultured with PE anti-mouse CD80 (Biolegend, 104708) antibody at 4° C. for 30 minutes. Next, they were washed with PBS containing 2% FBS and analyzed using a Cytoflex flow cytometer. All data were analyzed using Kaluza software.

[0217] As shown in FIGS. 15a and 15b, it was confirmed that IEP-01i' induced the activation of macrophages as well as LPS, a positive control group.

##### Experimental Example 9.2. Confirmation of Macrophage Differentiation by Exosomes

[0218] In order to confirm macrophage differentiation by ciMSC-derived exosomes, Raw264.7 cells, a mouse-derived macrophage cell line, were pretreated with 20 ng/ml IL-4 for 24 hours to induce the differentiation into M2 macrophages. Thereafter, the cells were treated with 10 ng/ml of LPS or 1000 ptc/ml of IEP-01i' (2-chem-treated ciMSC-derived exosomes) for 24 hours. After treatment, the cells were washed with PBS and cultured with PE anti-mouse CD80 (Biolegend, 104708) antibody for 30 minutes at 4° C., and then they were washed with PBS containing 2% FBS and analyzed using a Cytoflex flow cytometer. All data were analyzed using Kaluza software.

[0219] As shown in FIGS. 15c and 15d, it was confirmed that when treated with IL-4, differentiation into M2 macrophages occurred, and M1 macrophage markers were decreased, whereas when treated with IEP-01i', the expression of CD80, an M1 macrophage marker, was strongly induced, as when treated with LPS.

[0220] These results indicate that differentiation into M2 macrophages is inhibited by IEP-01i' and differentiation into M1 macrophages is induced.

#### Experimental Example 10. Confirmation of Dendritic Cell Activation by ciMSC-Derived Exosomes

[0221] In order to confirm dendritic cell activation by ciMSC-derived exosomes, fluorescence-

labeled ciMSC-derived exosomes were prepared for each treated group as in Experimental Example 8 above.

[0222] Immature dendritic cells derived from human peripheral blood (Stem cell technologies, #70041) were treated with labeled naive or IEP-01i' exosomes or unlabeled naive or IEP-01i' exosomes at a concentration of 3000 particles/cell, cultured in a CO.sub.2 incubator at 37° C. for 24 hours, and then washed twice with PBS. As a positive control group, immature dendritic cells were treated with 1 µg/mL of LPS for 24 hours to activate dendritic cells. In order to confirm dendritic cell activation, the cells were cultured with fluorescence-labeled antibodies at 4° C. for 30 minutes. Thereafter, they were washed with PBS containing 2% FBS and analyzed using a Cytoflex flow cytometer. All data were analyzed using Kaluza software.

[0223] APC anti-human CD11c (Biolegend, 301614), PE anti-human CD80 (Biolegend, 305208), PE anti-human CD86 (Biolegend, 374206), PE anti-human HLA-A,B,C (MHC class I) (Biolegend, 311406), PE anti-human HLA-DR, DP, DQ (MHC class II) (361716) antibodies were used for the analysis.

[0224] As shown in FIGS. **16a** to **16d**, PKH-positive dendritic cells were confirmed in the group treated with PKH-labeled exosomes.

[0225] As shown in FIGS. **16e** and **16f**, it was confirmed that CD80, a dendritic cell activation marker, was expressed more in the dendritic cell group treated with IEP-01i' exosomes than in the untreated dendritic cells. In particular, it was confirmed that the expression of CD80 was significantly increased specifically in the dendritic cells labeled with PKH, i.e., the dendritic cells that had absorbed the exosomes.

[0226] In addition, as shown in FIGS. **16g** and **16h**, it was confirmed that CD86, a dendritic cell activation marker, was expressed more in the dendritic cell group treated with IEP-01i' exosomes than in the untreated dendritic cells. In particular, it was confirmed that the expression of CD86 was significantly increased specifically in the dendritic cells labeled with PKH, i.e., the dendritic cells that had absorbed the exosomes. In addition, it was confirmed that CD86 was expressed more highly in the dendritic cells treated with IEP-01i' compared to the dendritic cells treated with naive exosomes.

[0227] As shown in FIGS. **16i** and **16j**, it was confirmed that MHC class I, an antigen presenting molecule, was expressed more highly in the dendritic cell group treated with IEP-01i' exosomes compared to the untreated dendritic cells. In particular, it was confirmed that the expression of MHC class I was significantly increased specifically in the dendritic cells labeled with PKH, i.e., the dendritic cells that had absorbed the exosomes.

[0228] As shown in FIGS. **16k** and **16l**, it was confirmed that MHC class II, an antigen presenting molecule, was expressed more highly in the dendritic cell group treated with IEP-01i' exosomes compared to the untreated dendritic cells. In particular, it was confirmed that the expression of MHC class II was significantly increased specifically in the dendritic cells labeled with PKH, i.e., the dendritic cells that had absorbed the exosomes. In addition, it was confirmed that significantly more MHC class II was expressed in the dendritic cells treated with IEP-01i' compared to the dendritic cells treated with naive exosomes.

#### Experimental Example 11. Analysis of Cytokines of ciMSC-Derived Exosomes

[0229] In order to analyze cytokines contained in ciMSC-derived exosomes, the ciMSC-derived exosome sample of Experimental Example 7.1 above was prepared at a concentration of  $1 \times 10^{10}$  ptc/ml. Thereafter, 1% Triton X-100 was added and treated at room temperature for 10 minutes to lyse the exosomes. Next, the cytokines inside the exosomes were identified and the amount of cytokines was quantified using the Legendplex human essential immune response panel (Biolegend, 740930). The analysis was performed according to the manufacturer's manual, and the analysis of all samples was performed in triplicate.

[0230] As shown in FIGS. **17a** to **17e**, it was confirmed that IEP-01i' contained significantly more IP-10, IL-6, MCP-1, and IL-8 than naive exosomes. On the other hand, the amount of TGF-β1 was

much smaller than that of naive exosomes.

[0231] These results indicate that IEP-01i' contains cytokine molecules that can activate the chemoattraction of dendritic cells or macrophages. In addition, these results indicate that it contains less TGF- $\beta$ 1, which promotes the growth of cancer cells.

[0232] Therefore, it is predicted that the growth of cancer cells can be suppressed through the infiltration and activation of innate immune cells by cytokines of ciMSC-derived exosomes.

#### Experimental Example 12. Animal Experiment for Colorectal Cancer Cancer Using ciMSC-Derived Exosomes

##### Experimental Example 12.1. Single Administration of ciMSC-Derived Exosomes

[0233] In order to confirm the anticancer effect of single administration of ciMSC-derived exosomes, the exosomes were administered alone at various doses to the colorectal cancer cancer mice. The colorectal cancer cancer cells were constructed as in Example 6.1. above, and the colorectal cancer cancer mice were prepared as in Example 6.2. above.

[0234] During the acclimatization period, healthy animals were selected and cell line inoculation was performed. Thereafter, when the tumor size of the site transplanted with the cell line reached about 100 mm.<sup>sup.3</sup>, the mice were grouped according to tumor size so that the tumor size of each group was distributed as evenly as possible. Administration was performed as shown in FIG. **18a**. Specifically, the test substance was administered intravenously at a low dose ( $5 \times 10^8$  ptc/200  $\mu$ l), a mid dose ( $1.5 \times 10^9$  ptc/200  $\mu$ l), and a high dose ( $5 \times 10^9$  ptc/200  $\mu$ l) once daily or once every two days, for a total of six times. The occurrence of tumors was checked three times a week from the date of group separation or from the start date of the test substance administration (Day 0), and the tumor size was measured. The tumor size was measured as in Example 6.4. above.

[0235] As shown in FIG. **18b**, it was confirmed that IEP-01i' exhibited an anticancer effect against CT26 in a dose-dependent manner regardless of the treatment interval. In addition, it was confirmed that IEP-01i' exhibited significant anticancer effects in the CT26 tumor model as a single administration.

[0236] In addition, as shown in FIGS. **18c** to **18i**, it was confirmed that IEP-01i' exhibited an anticancer effect against CT26 in a dose-dependent manner regardless of the treatment interval. In particular, it was confirmed that some subjects in the high-dose treated group showed complete response.

##### Experimental Example 12.2. Combined Administration of ciMSC-Derived Exosomes

[0237] In order to confirm the anticancer effect when exosomes were administered alone or in combination with an immune checkpoint inhibitor, the colorectal cancer cancer mice were treated with an anti-PD-1 antibody, IEP-01, and IEP-01i alone or in combination. The experiment was performed in the same manner as in Experimental Example 12.1 above.

[0238] Administration was performed as shown in FIG. **19a**. Each test animal was treated three times a week, for a total of six times by administering intraperitoneally the anti-PD-1 antibody at 5 mg/kg and administering intravenously IEP-01 (WJ-MSC-derived exosome) or IEP-01i (ciMSC-derived exosome) at  $5 \times 10^8$  ptc/200  $\mu$ l. The occurrence of tumors was checked three times a week from the date of group separation or from the start date of the test substance administration (Day 0), and the tumor size was measured.

[0239] As shown in FIG. **19b**, some anticancer effects were observed in the group treated with IEP-01 or IEP-01i alone. In addition, it was confirmed that the anticancer effects were significant in the group treated with IEP-01 or IEP-01i in combination with an anti-PD-1 antibody. In some mouse subjects, complete response was observed by each treatment, and in particular, the most complete response (CR) was observed in the group treated with IEP-01 or IEP-01i in combination with an anti-PD-1 antibody. In the mouse models where complete response was observed, when CT26 cancer cells were re-injected, the cancer cells were observed to not grow, which means that immunological memory was formed in the subjects treated with each treatment.

[0240] In addition, as shown in FIGS. **19c** to **19i**, as a result of monitoring the tumor growth of

each subject in a CT26 colorectal cancer animal model, it was confirmed that there was a clear tumor growth inhibition, although there were differences among subjects.

**Experimental Example 13. Animal Experiment for Melanoma Using ciMSC-Derived Exosomes**  
[0241] In order to confirm the anticancer effect when ciMSC-derived exosomes were administered alone or in combination, the melanoma mice were treated with an anti-PD-1 antibody and IEP-01i alone or in combination.

[0242] Melanoma mice were constructed in the same manner as in Example 6.1. above except that the frozen mouse tumor cell line (B16F10 cell line) was used. In addition, experiments were prepared in the same manner as in Example 6.2. above.

[0243] During the acclimatization period, healthy animals were selected and cell line inoculation was performed. Thereafter, when the tumor size of the site transplanted with the cell line reached about 100 mm<sup>sup.3</sup>, the mice were grouped according to tumor size so that the tumor size of each group was distributed as evenly as possible. Administration was performed as shown in FIG. 20a. Each test animal was treated three times a week for 2 weeks, for a total of six times by administering intraperitoneally the anti-PD-1 antibody at 5 mg/kg and administering intravenously IEP-01i at 5×10<sup>sup.8</sup> ptc/200 1 μl. The occurrence of tumors was checked three times a week from the date of group separation or from the start date of the test substance administration (Day 0), and the tumor size was measured.

[0244] As shown in FIG. 20b, it was confirmed that when treated with IEP-01i and an anti-PD-1 antibody, respectively, the tumor size of B16F10 was reduced, and when treated in combination, tumor inhibition was more significant than in the group treated alone.

[0245] In addition, as shown in FIGS. 20c to 20i, in the groups treated with IEP-01i and an anti-PD-1 antibody alone or in combination, a number of subjects were observed to have slowed tumor growth rates. In addition, it was confirmed that a large number of mice (3/7) died in the group treated with PBS, whereas the number of dead subjects was lower in the groups treated with IEP-01i and an anti-PD-1 antibody alone or in combination (IEP-01i alone; 2/7, anti-PD-1 antibody alone; 1/7, IEP-01i in combination with anti-PD-1 antibody; 1/7).

**Experimental Example 14. Animal Experiment for Pancreatic Cancer Using ciMSC-Derived Exosomes**

[0246] In order to confirm the anticancer effect when ciMSC-derived exosomes were administered alone or in combination, the pancreatic cancer mice were treated with an anti-PD-1 antibody and IEP-01i alone or in combination.

[0247] Pancreatic cancer mice were prepared in the same manner except that the frozen mouse tumor cell line (Pan-02 cell line) produced as follows was used.

[0248] For cell line preparation, 1 vial of the frozen mouse tumor cell line (Pan-02 cell line) was placed in a cell culture flask containing RPMI1640 medium containing 10% heat-inactivated FBS (fetal bovine serum, Gibco, 10082-147) and cultured in a 5% CO<sub>2</sub> incubator at 37° C. The cells were washed with PBS, and 2.5% Trypsin-EDTA (Gibco, 15090) was diluted 10 times. Thereafter, the cell line was separated by adding it and then subjected to centrifugation (1,000 rpm, 5 minutes), and the supernatant was discarded, and then the cell suspension was obtained with fresh medium. The viability was confirmed using a microscope, and then the cell line was prepared by diluting it in a 11 mixture of medium and Matrigel at a concentration of 5.0×10<sup>sup.6</sup> cells/mL.

[0249] During the acclimatization period, healthy animals were selected and cell line inoculation was performed. Thereafter, when the tumor size of the site transplanted with the cell line reached about 100 mm<sup>sup.3</sup>, the mice were grouped according to tumor size so that the tumor size of each group was distributed as evenly as possible. Administration was performed as shown in FIG. 20a. Each test animal was treated three times a week for 2 weeks, for a total of six times by administering intraperitoneally the anti-PD-1 antibody at 15 mg/kg and administering intravenously IEP-01i' at 1×10<sup>sup.10</sup> ptc/200 μl. The occurrence of tumors was checked three times a week from the date of group separation or from the start date of the test substance

administration (Day 0), and the tumor size was measured.

[0250] As shown in FIGS. 21*b* to 21*f*, it was confirmed that there was little difference in tumor growth rate in the group treated with an anti-PD-1 antibody compared to the negative control group treated with vehicle (PBS), whereas the tumor growth rate of pancreatic cancer was significantly slowed in the group treated with IEP-01i' alone. In addition, it was confirmed that pancreatic cancer was inhibited the most in the group treated with IEP-01i' in combination with an anti-PD-1 antibody.

[0251] These results indicate that IEP-01i' can effectively inhibit tumor growth even in solid tumors such as pancreatic cancer that do not respond well to immune checkpoint inhibitors such as anti-PD-1 antibodies.

#### Experimental Example 15. Evaluation of Poly IC Inclusion in IEP-01

[0252] After purification of exosomes (IEP-01), samples before and after the purification process were analyzed using HPLC to determine whether poly IC peaks were detected.

[0253] As shown in FIGS. 22*a* to 22*c*, in the case of poly IC simply mixed in the medium, it was confirmed that all of poly IC was removed during the purification process, but in the case of IEP-01, poly IC was still detected even after the purification process.

[0254] These results indicate that poly IC is contained within IEP-01, and the comparison results with naive exosomes also indicate that poly IC exists only in IEP-01.

#### Experimental Example 16. Analysis of miRNAs Present in ciMSC-Derived Exosomes Treated with Chemical Substance Cocktail

[0255] miRNAs present in exosomes were analyzed using MacroGen's Affymetrix miRNA 4.0 service.

[0256] Specifically, miRNAs contained in exosome samples were extracted, the library was constructed, and then sequencing was performed. By comparing the resulting sequence information with the existing miRbase database, the miRNAs that are relatively more expressed in each exosome sample were analyzed, and the results are shown in FIG. 23. Ctrl\_Exo is an exosome obtained from untreated ciMSCs, 3 chem Exo is an exosome obtained from ciMSCs that were treated with 3 chem, and 2 chem Exo is an exosome obtained from ciMSCs that were treated with 2 chem.

[0257] The results of measuring the types of miRNAs present in each exosome and their expression intensities are shown in Table 4 below.

TABLE-US-00004	TABLE 4	Transcript ID (Array Design)	Ctrl Exo	3 chem Exo	2 chem Exo	hsa-miR-574-5p
	2.431340	10.242110	11.253710	hsa-miR-6086	4.905830	10.407030
	1.620650	hsa-miR-4701-3p	3.464640	8.666940	9.709270	hsa-miR-3149
	0.854740	6.039780	8.705470	hsa-miR-4743-5p	6.657570	11.590950
	11.714050	hsa-miR-8075	5.973260	10.838260	11.220240	hsa-miR-6880-5p
	5.401840	10.000900	5.018530	hsa-miR-3148	1.299280	5.893540
	7.617760	hsa-miR-6780b-5p	6.760150	11.072200	9.817130	hsa-miR-6846-5p
	6.528900	10.589760	10.657420	hsa-mir-320e	6.245890	10.228630
	10.101240	hsa-miR-32-3p	1.483410	5.424420	7.875170	hsa-miR-642a-3p
	7.378470	10.459880	10.819200	hsa-miR-4505	10.281990	13.026020
	12.986160	hsa-miR-595	2.434340	5.102860	8.189200	hsa-miR-4487
	4.401570	6.858980	5.015790	hsa-miR-4721	6.131810	8.575690
	7.869180	hsa-miR-7151-3p	3.061450	5.424420	5.955770	hsa-miR-4484
	10.642280	12.915840	11.641950	hsa-miR-642b-3p	6.809470	9.076000
	10.405250	ENSG00000239154	3.881070	6.112830	5.860120	hsa-miR-4706
	7.523010	9.646450	7.645370	hsa-miR-6126	10.413740	12.388690
	12.388690	hsa-miR-378h	3.627520	5.496800	4.926410	hsa-miR-6849-5p
	3.703310	5.424420	7.195840	hsa-miR-7844-5p	1.371450	3.089640
	6.132980	hsa-miR-4535	4.702010	6.373170	6.373170	hsa-mir-9-1
	2.941450	4.552650	5.270960	hsa-miR-4459	8.104580	9.663110
	8.935170	hsa-miR-4443	5.790840	7.230830	12.209800	hsa-miR-6732-5p
	9.461700	10.722030	11.155110	hsa-miR-3064-5p	2.513040	3.733250
	4.466610	hsa-miR-3178	10.666070	11.761590	12.505970	hsa-miR-3135b
	10.625810	11.636710	11.807040	hsa-miR-4717-		

3p 3.551030 4.180980 7.376090 hsa-miR-206 1.761700 2.084930 4.191640 hsa-miR-4529-3p  
2.432770 2.432770 6.025090 hsa-miR-3613-5p 2.030240 1.363560 4.762030

## Claims

1. An extracellular vesicle isolated from a human-derived cell, wherein the extracellular vesicle i) comprises at least one miRNA selected from the group consisting of NovelmiRNA-67, hsa-miR-199a-3p, NovelmiRNA-298, hsa-miR-145-5p, NovelmiRNA-281, NovelmiRNA-109, hsa-miR-432-5p, NovelmiRNA-230, NovelmiRNA-88, hsa-miR-200b-3p, NovelmiRNA-169, hsa-miR-200a-3p, hsa-miR-487b-3p, hsa-miR-214-3p, NovelmiRNA-208, NovelmiRNA-164, NovelmiRNA-225, NovelmiRNA-174, hsa-miR-708-5p, hsa-miR-124-3p, NovelmiRNA-150, hsa-miR-135a-5p, NovelmiRNA-64, NovelmiRNA-92, NovelmiRNA-168, NovelmiRNA-22, NovelmiRNA-35, NovelmiRNA-161, hsa-miR-3940-3p, hsa-miR-219a-3p, hsa-miR-212-3p, NovelmiRNA-76, hsa-miR-323a-3p, hsa-miR-654-3p, hsa-miR-204-5p, hsa-miR-136-3p, NovelmiRNA-39, NovelmiRNA-175, hsa-miR-134-5p, hsa-miR-454-3p, NovelmiRNA-286, hsa-miR-542-3p, hsa-miR-433-3p, hsa-miR-574-5p, hsa-miR-6086, hsa-miR-4701-3p, hsa-miR-3149, hsa-miR-4743-5p, hsa-miR-8075, hsa-miR-6880-5p, hsa-miR-3148, hsa-miR-6780b-5p, hsa-miR-6846-5p, hsa-miR-320e, hsa-miR-32-3p, hsa-miR-642a-3p, hsa-miR-4505, hsa-miR-595, hsa-miR-4487, hsa-miR-4721, hsa-miR-7151-3p, hsa-miR-4484, hsa-miR-642b-3p, ENSG00000239154, hsa-miR-4706, hsa-miR-6126, hsa-miR-378h, hsa-miR-6849-5p, hsa-miR-7844-5p, hsa-miR-4535, hsa-miR-9-1, hsa-miR-4459, hsa-miR-4443, hsa-miR-6732-5p, hsa-miR-3064-5p, hsa-miR-3178, hsa-miR-3135b, hsa-miR-4717-3p, hsa-miR-206, hsa-miR-4529-3p, and hsa-miR-3613-5p, and ii) comprises at least one mRNA selected from the group consisting of MED13L, lnc-TOMM20L-1, lnc-CDK5R1-1, ETV3, SLC35G1, SMARCA4, ACAT2, ADAM12, ARFGEF1, CELF3, DNAJB5, lnc-WRNIP1-2, ACTL8, lnc-TM2D3-2, GCN1L1, LOC101929337, FAM217A, XLOC\_l2\_013467, SNHG4, NUDT13, MCTS1, ATG4D, NPEPL1, YEATS2, SPEN, GGT6, lnc-RP11-770J1.4.1-1, MIAT, lnc-HMCN1-2, RETN, lnc-EXT1-2, LIN54, C17orf74, SLC12A2, TTC28, ADRA2A, NLGN2, lnc-FBXO25-4, LOC100506603, LOC399886, RCC2, KIAA0040, HOXB9, JMJD8, LINC01146, lnc-GIT1-1, VRTN, NHP2, KIF26A, HAUS5, lnc-SOD2-2, APOL3, lnc-POLR2L-1, lnc-SUPT3H-1, lnc-XRN2-3, RTP2, TERT, TNFRSF12A, LOC645427, lnc-CTR9-5, LOC100507420, OPA3, XLOC\_l2\_014245, lnc-NR5A1-1, DMTN, SMPD4, TXLNB, SPRR1A, DISP1, LOC100130285, lnc-SLC17A9-1, lnc-SELV.1-1, XLOC\_l2\_000018, lnc-ROM1-2, lnc-RABEPK-1, lnc-RRP8-1, lnc-RP11-234B24.6.1-1, lnc-TMEM189-UBE2V1-2, LAMTOR2, BTK, lnc-BSND-1, C2orf71, lnc-C17orf63-2, lnc-CHGA-1, ABCD1, NCAN, C9orf106, AGAP2, EMC6, CD86, LOC100129175, lnc-PHYHD1-1, GIGYF2, RNF151, BAGE, ADRA2C, lnc-SLC43A1-1, ENTPD8, NYAP1, lnc-GLIPR1L1-1, CCNT2-AS1, NAV1, TBX21, GNAZ, DNM3OS, PRKAG2-AS1, lnc-MFSD9-4, lnc-RPGRIP1-2, ABCC6, LRFN3, lnc-COL1A1-2, lnc-PPAPDC3-2, CTU1, lnc-PABPC4-1, LOC100507165, LOC102724861, SPTSSB, lnc-C20orf96-4, DCDC5, lnc-CRYL1-1, LOC100506114, SHARPIN, ASMTL-AS1, HMOX1, SHARPIN, LOC101928207, TRAIP, lnc-ANTXRL-2, LINC01135, BZRAP1, LOC101926983, WDR6, C19orf81, NAMA, LIM2, FAM155A-IT1, lnc-RPP30-2, ZNF264, MALAT1, HTT-AS, ZDHHC22, SEPHS2, ABTB1, lnc-RP11-791J7.2.1-1, CCDC28B, LOC284263, lnc-PPIC-2, CDC42BPB, C9orf173-AS1, C9orf139, FGD5, SP6, RCOR2, lnc-AC016251.1-2, lnc-PRKAA2-1, RANBP3, lnc-SH2B3-1, XLOC\_l2\_011901, CACNA1H, LINC01314, lnc-VAPB-1, ARHGAP23, ATG9A, GM2A, XLOC\_l2\_013547, lnc-SLC25A26-1, TMEM240, CLSTN3, ATG16L2, MMP11, TSSK3, lnc-SOX6-1, EDEM3, LOC101927210, C14orf169, EBLN2, lnc-XRN1-1, lnc-FTSJD2-1, MDFI, LOC102546226, SLC5A10, SENP7, lnc-C17orf97-4, ARNTL, lnc-RTL1-1, ZNF713, XLOC\_l2\_005871, QSOX1, SP2-AS1, ARSA, PHF7, GS1-24F4.2, LRIT1, PLLP, SLC4A11, C11orf95, FAM110B, IER5, LINC01508, lnc-GTPBP8-1, LOC400958, WFDC21P, lnc-CPXM2-2, P2RX6, SLC22A7, lnc-PPP2R2C-1, lnc-GATA5-2, MARVELDI, lnc-TRIM41-3, PRSS8,

SEMA6C, SLC48A1, RPL28, BGN, BZW1, lnc-C6orf221-2, SLC20A2, HYALP1, SNAR-G1, SEC22A, TIMM8B, ATAD1, FNDC7, B3GNT8, STARD7-AS1, TMEM100, LARP6, HELZ2, TMX2, lnc-EPHB6-1, PSMC2, lnc-LINC00273-1, TNFRSF25, BCL3, LOC101929450, LOC102724416, FLII, WAC, PPP6R1, LOC100507377, LRRFIP2, CCNT2-AS1, lnc-SCAMP5-1, BRD1, lnc-ELAVL4-3, CHRDL1, lnc-FUT8-1, lnc-DNAJC7-1, LINC01264, LMO2, LINC01197, LOC101927533, REP15, C1orf226, lnc-STEAP1B-1, SNORD3B-1, lnc-TMEM135-2, lnc-FAM160A1-1, GOLGA2P5, TMEM132C, GPX3, lnc-DCAF4L2-2, XLOC\_12\_014217, PPT1, RPA4, KCTD5, lnc-UQCRFS1-9, MTUS2-AS1, CHKA, TBC1D31, SNORD38A, lnc-RP11-322L20.1.1-7, RAD51, MEIOB, lnc-ADAMTS14-1, PCDHA13, and NOS2, and iii) comprises at least one cytokine selected from the group consisting of IL-6, IL-8, MCP-3, MCP-1, IP-10, IL-1 $\beta$ , and RANTES.

**2.** The extracellular vesicle according to claim 1, wherein the extracellular vesicle comprises at least one selected from the group consisting of zymosan, polyinosinic-polycytidylic acid (Poly I:C), and monophosphoryl lipid A (MPLA).

**3.** The extracellular vesicle according to claim 1, wherein the human-derived cell includes a cell, a cell line, or a stem cell derived from human tissue.

**4.** The extracellular vesicle according to claim 3, wherein the stem cell is a mesenchymal stem cell, a hematopoietic stem cell, a neural stem cell, an embryonic stem cell, or an induced pluripotent stem cell.

**5.** The extracellular vesicle according to claim 4, wherein the mesenchymal stem cell is derived from umbilical cord, umbilical cord blood, Wharton's jelly, bone marrow, fat, muscle, nerve, skin, amniotic membrane, tooth, hair root cell, or placenta, or is differentiated from an induced pluripotent stem cell.

**6.** The extracellular vesicle according to claim 1, wherein the extracellular vesicle i) is positive for at least one marker selected from the group consisting of CD9, CD63, CD81, and TSG101, and ii) is negative for at least one marker selected from the group consisting of GM130 and Calnexin.

**7.** An extracellular vesicle isolated from a human-derived cell treated with at least one selected from the group consisting of zymosan, Poly I:C, and monophosphoryl lipid A (MPLA).

**8.** The extracellular vesicle according to claim 7, wherein the human-derived cell includes a cell, a cell line, or a stem cell derived from human tissue.

**9.** The extracellular vesicle according to claim 8, wherein the stem cell is a mesenchymal stem cell, a hematopoietic stem cell, a neural stem cell, an embryonic stem cell, or an induced pluripotent stem cell.

**10.** The extracellular vesicle according to claim 9, wherein the mesenchymal stem cell is derived from umbilical cord, umbilical cord blood, Wharton's jelly, bone marrow, fat, muscle, nerve, skin, amniotic membrane, tooth, hair root cell, or placenta, or is differentiated from an induced pluripotent stem cell.

**11.** A pharmaceutical composition for preventing or treating cancer, comprising the extracellular vesicle according to claim 1 as an active ingredient.

**12.** The pharmaceutical composition for preventing or treating cancer according to claim 11, wherein the cancer is any one selected from the group consisting of gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myeloid leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer, and lymphoma.

**13.** A pharmaceutical composition for combination administration for preventing or treating cancer, comprising the extracellular vesicle according to claim 1 and an immune anticancer agent as active ingredients.

**14.** The pharmaceutical composition for combination administration for preventing or treating cancer according to claim 13, wherein the immune anticancer agent is any one selected from the group consisting of an immune checkpoint inhibitor, a chimeric antigen receptor-T cell (CAR-T), a



- T cell receptor modified T cell (TCR-T), a tumor infiltrating lymphocyte (TIL), a bispecific T-cell engager (BiTE), a tumor vaccine, and an oncolytic virus.
- 15.** The pharmaceutical composition for combination administration for preventing or treating cancer according to claim 14, wherein the immune checkpoint inhibitor is any one selected from the group consisting of an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-B7-H4 antibody, an anti-HVEM antibody, an anti-TIM3 antibody, an anti-GAL9 antibody, an anti-LAG3 antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-BTLA antibody, and an anti-TIGIT antibody.
- 16.** A method for preparing extracellular vesicles, comprising: a) culturing human-derived cells in a serum-free cell culture medium supplemented with Poly I:C and monophosphoryl lipid A (MPLA); b) obtaining a culture solution during the culturing process; and c) filtering the obtained culture solution to remove particles larger than 200 nm and then isolating the extracellular vesicles.
- 17.** The method for preparing extracellular vesicles according to claim 15, wherein the serum-free cell culture medium further comprises zymosan.
- 18.** A method for preventing or treating cancer, comprising administering the extracellular vesicle according to claim 1 to a subject.
- 19.** Use of the extracellular vesicle according to claim 1 for the prevention or treatment of cancer.
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