

microarray.

[Best Mode for Implementation of the Invention]

Hereinafter, the present disclosure will be described in more detail.

5 As used herein, the term "about" refers to quantity, level, value, number, frequency, percentage, dimension, size, amount, weight, or length that vary by 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 degrees with respect to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight, or length.

 As used herein, the term “artificially engineered” is a term used to distinguish
10 substances, molecules and the like that have a composition that is already present in the natural world, and means that artificial modifications have been made to the substances, molecules and the like. For example, the expression “artificially engineered gene” refers to a gene in which artificial modifications have been made to the composition of genes that are present in the natural world. In addition, the above term includes all meanings that may be recognized by a
15 person skilled in the art, and may be appropriately interpreted depending on the context.

 As used herein, the term “wild-type” refers to that a gene including a naturally occurring base sequence and a protein expressed from the gene have normal functional characteristics. Wild-type genes have a form in which no natural or artificial mutations have occurred and are most frequently observed in the population. When the term “wild-type” is
20 used herein in contrast to artificially engineered genes and/or artificially engineered cells, this may be interpreted to mean a gene including an artificially engineered gene and/or a

generated from genome assembly sequences or transcripts. The alignment file was used to assemble transcripts, estimate abundance, and detect differential expression of genes. Differentially expressed genes were determined based on counts from unique and multiple alignments using coverage in Bedtools (Quinlan AR, 2010). Using Bioconductor (Gentleman et al., 2004), RC (Read Count) data was processed based on a TMM+CPM normalization method using EdgeR within R (R development Core Team, 2020). Gene classification was based on searches by the DAVID (<http://david.abcc.ncifcrf.gov/>) and Medline databases (<http://www.ncbi.nlm.nih.gov/>) engines.

FIG. 12 is a table showing a list of genes with a p-value of less than 0.05 and a normalized data (log 2) value of 4 or more as a result of RNA-seq (QuantSeq) analysis.

Antibody microarray

Antibody array analysis of gene-edited mesenchymal stem cells was performed by Full Moon Biosystems. Cell lysate was prepared using Protein Extraction Kit (Full Moon BioSystems). The clear supernatant of the lysate was isolated, biotinylated, and incubated using an antibody microarray (Phospho Explorer Antibody Arrays, Full Moon BioSystems) of phosphorylated proteins for 2 hours at room temperature. Array slides were washed with wash buffer (Full Moon BioSystems) and rinsed with deionized water. Slides were then incubated with Cy3-Streptavidin for 45 min at room temperature and then washed, rinsed, and dried. Arrays were scanned on a GenePix Array Scanner (Molecular Devices). Image quantification was performed with GenePix Pro (Molecular Devices). Signal intensity data for each point on the array was extracted from the array image. Since two replicates are

[CLAIMS]

[Claim 1]

Artificially engineered mesenchymal stem cells comprising an artificially engineered F3 gene, wherein the artificially engineered F3 gene is different from the F3 gene sequence of a wild-type mesenchymal stem cell, the artificially engineered F3 gene includes one or more indels in the nucleic acid sequence, and an expression level of CD142 on the surface of the artificially engineered mesenchymal stem cell is reduced compared to the wild-type mesenchymal stem cell, thereby improving hemocompatibility.

[Claim 2]

The artificially engineered mesenchymal stem cell of claim 1, wherein the indel is located within a protospacer-adjacent motif (PAM) sequence in the first, second, third, fourth or sixth exon region of the F3 gene, or within a contiguous sequence of 5 to 50 nucleotides adjacent to the 5' or 3' end of the PAM sequence.

[Claim 3]

The artificially engineered mesenchymal stem cell of claim 1, wherein a sequence of the artificially engineered F3 gene does not include one or more sequences selected from the group consisting of SEQ ID NOs: 1 to 43.

[Claim 4]

The artificially engineered mesenchymal stem cell of claim 1, wherein, in the artificially engineered mesenchymal stem cell, an mRNA transcribed from the artificially engineered F3 gene has a lower mRNA expression level or a different sequence compared to

the mRNA expression level transcribed from the F3 gene of the wild-type mesenchymal stem cell.

[Claim 5]

The artificially engineered mesenchymal stem cell of claim 1, wherein the artificially engineered mesenchymal stem cells is derived from fat, bone marrow, umbilical cord, placenta, amniotic fluid, amniotic membrane, tissue, umbilical cord blood, or perinatal tissue.

[Claim 6]

A composition for preparing hemocompatible mesenchymal stem cells, the composition comprising:

a guide nucleic acid including a guide sequence capable of targeting a target sequence of the F3 gene of a mesenchymal stem cell, or a nucleic acid encoding the same; and

an editor protein or a nucleic acid encoding the same.

[Claim 7]

The composition of claim 6, wherein the target sequence is one or more sequences selected from SEQ ID NO: 1 to SEQ ID NO: 43.

[Claim 8]

The composition of claim 6, wherein the composition includes the editor protein and the guide nucleic acid in the form of ribonucleoprotein (RNP).

[Claim 9]

The composition of claim 6, wherein the composition includes a nucleic acid encoding the editor protein and a nucleic acid encoding the guide nucleic acid in the form of one or more vectors.

[Claim 10]

5 The composition of claim 9, wherein the vector is selected from the group consisting of plasmid, retrovirus, lentivirus, adenovirus, adeno-associated virus, vaccinia virus, poxvirus, and herpes simplex virus.

[Claim 11]

A preparation method for hemocompatible stem cells, the method comprising:

10 (1) introducing a composition for preparing hemocompatible mesenchymal stem cells including a guide nucleic acid capable of targeting a target sequence of F3 gene or a nucleic acid encoding the same; and an editor protein or a nucleic acid encoding the same into isolated mesenchymal stem cells; and

 (2) editing the F3 gene to reduce or suppress the expression or activity of CD142 by
15 generating an indel in the target sequence of the F3 gene located in the genome of the mesenchymal stem cell.

[Claim 12]

The method of 11, wherein the target sequence is one or more sequences selected from SEQ ID NO: 1 to SEQ ID NO: 43.

20 [Claim 13]

The method of 11, wherein the composition includes a nucleic acid encoding the editor protein and a nucleic acid encoding the guide sequence in the form of one or more vectors.

[Claim 14]

5 The method of 11, wherein a CRISPR/Cas9 complex including Streptococcus pyogenes-derived Cas9 protein and guide RNA that may target the target sequence of the F3 gene is contacted with the target sequence of F3 gene located within the genome of the mesenchymal stem cell, thereby generating an indel within the target sequence.

[Claim 15]

10 A cell therapeutic agent for vascular administration comprising the artificially engineered mesenchymal stem cells of any one of claims 1 to 5 as an active ingredient.

[Claim 16]

The cell therapeutic agent of claim 15, wherein the cell therapeutic agent for vascular administration is intended to treat one type of disease selected from the group consisting of myocardial infarction, heart failure, ischemic cardiomyopathy, myocarditis, ischemic enteritis, inflammatory bowel disease, ulcerative colitis, Crohn's disease, acute cholecystitis, acute cholangitis, chronic cholecystitis, acute pancreatitis, chronic pancreatitis, acute nephritis, chronic nephritis, acute renal failure, chronic renal failure, pneumonia, interstitial pneumonia, idiopathic interstitial pneumonia, exfoliative interstitial pneumonia, acute interstitial pneumonia, non-specific interstitial pneumonia, drug-induced lung disease, acute respiratory distress syndrome, chronic obstructive pulmonary disease, cerebral palsy syndrome including pediatric cerebral palsy, amyotrophic lateral sclerosis (ALS), polyneuropathy, spinal muscular

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atrophy, acute transverse myelitis, stroke, multiple sclerosis, peripheral artery disease (PAD), thromboangiitis obliterans (Buerger's disease), Kawasaki disease (KD), and graft versus host disease (GVHD).

[Claim 17]

- 5 A pharmaceutical composition comprising the artificially engineered mesenchymal stem cells of any one of claims 1 to 5 as an active ingredient.

[Claim 18]

The pharmaceutical composition of claim 17, wherein the composition is administered intravascularly.

- 10 [Claim 19]

The pharmaceutical composition of claim 17, wherein the composition is intended for use in the prevention or treatment of one type of disease selected from the group consisting of myocardial infarction, heart failure, ischemic cardiomyopathy, myocarditis, ischemic enteritis, inflammatory bowel disease, ulcerative colitis, Crohn's disease, acute cholecystitis, acute cholangitis, chronic cholecystitis, acute pancreatitis, chronic pancreatitis, acute nephritis, chronic nephritis, acute renal failure, chronic renal failure, pneumonia, interstitial pneumonia, idiopathic interstitial pneumonia, exfoliative interstitial pneumonia, acute interstitial pneumonia, non-specific interstitial pneumonia, drug-induced lung disease, acute respiratory distress syndrome, chronic obstructive pulmonary disease, cerebral palsy syndrome including
15 pediatric cerebral palsy, amyotrophic lateral sclerosis (ALS), polyneuropathy, spinal muscular atrophy, acute transverse myelitis, stroke, multiple sclerosis, peripheral artery disease (PAD),
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thromboangiitis obliterans (Buerger's disease), Kawasaki disease (KD), and graft versus host disease (GVHD).