[CLAIMS]

[Claim 1]

Artificially engineered mesenchymal stems cells comprising an artificially engineered KEAP1 gene, wherein the artificially engineered Keap1 gene is different from KEAP1 gene sequence of a wild-type mesenchymal stem cell, the artificially engineered Keap1 gene includes one or more indels in the nucleic acid sequence, the indel is located within a protospacer-adjacent motif (PAM) sequence in the exon 2 region or exon 3 region of KEAP1 gene, or within a contiguous sequence of 5 to 50 nucleotides adjacent to the 5' or 3' end of the PAM sequence, and the artificially engineered mesenchymal stems cells has improved oxidative stress resistance.

[Claim 2]

The artificially engineered mesenchymal stem cells of claim 1, wherein a sequence of the artificially engineered Keap1 gene does not include one or more sequences selected from the group consisting of SEQ ID NOs: 1 to 15 and SEQ ID NOs: 49 to 56.

[Claim 3]

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

[Claim 4]

The artificially engineered mesenchymal stem cells of claim 1, wherein the artificially engineered stem cells have high survival in an oxidative stress environment.

[Claim 5]

The artificially engineered mesenchymal stem cells 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 mesenchymal stem cells with oxidative stress resistance, the composition comprising:

a guide nucleic acid including a guide sequence capable of targeting a target sequence of KEAP1 gene of mesenchymal stem cells, or a nucleic acid sequence encoding the same; and

an editor protein or a nucleic acid sequence encoding the same.

[Claim 7]

The composition of claim 6, wherein the target sequence is one or more sequences selected from the group consisting of SEQ ID NOs: 1 to 15 and SEQ ID NOs: 49 to 56.

[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 sequence encoding the editor protein and a nucleic acid sequence encoding the guide nucleic acid in the form of one or more vectors.

[Claim 10]

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 method of preparing stem cells with oxidative stress resistance, the method comprising:

(1) introducing a composition for preparing mesenchymal stem cells with oxidative stress resistance including a guide nucleic acid capable of targeting a target sequence of KEAP1 gene or a nucleic acid sequence encoding the same; and an editor protein or a nucleic acid sequence encoding the same into isolated mesenchymal stem cells; and

(2) editing KEAP1 gene to reduce or suppress the expression or activity of KEAP1 protein by generating an indel in a target sequence of KEAP1 gene located in the genome of the mesenchymal stem cells.

[Claim 12]

The method of claim 11, wherein the target sequence is one or more sequences

selected from the group consisting of SEQ ID NOs: 1 to 15 and SEQ ID NOs: 49 to 56.

[Claim 13]

The method of claim 11, wherein the indel is generated to be located within a protospacer-adjacent motif (PAM) sequence in the exon 2 region or exon 3 region of KEAP1 gene or within a contiguous sequence of 5 to 50 nucleotides adjacent to the 5’ or 3’ end of the PAM sequence.

[Claim 14]

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

[Claim 15]

The method of 11, wherein the indel is generated by contacting KEAP1 gene located within the genome of the mesenchymal stem cell with a CRISPR/Cas9 complex including Streptococcus pyogenes-derived Cas9 protein and guide RNA that may target the KEAP1 gene.

[Claim 16]

A pharmaceutical composition for preventing or treating an ischemic disease, comprising the artificially engineered mesenchymal stem cells of any one of claims 1 to 5 as an active ingredient.

[Claim 17]

The pharmaceutical composition of claim 16, wherein the ischemic disease is selected

from the group consisting of ischemic heart diseases, peripheral artery disease, critical limb ischemia (CLI), thromboangitis obliteran, diabetic peripheral angiopathy, osteonecrosis, mesenteric ischemia, ischemic colitis, ischemic enteritis, acute kidney injury, ischemia-reperfusion injury, ischemic hepatitis, ischemic pancreatitis, ischemic optic neuropathy, chronic obstructive pulmonary disease, acute respiratory distress syndrome (ARDS), COVID-19 infection, neonatal hypoxic-ischemic encephalopathy, or stroke.