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DESCRIPTION CN109295000A

Application of CREPT, an essential protein for pluripotent stem cell formation, in induced pluripotent stem cell therapy.

[0001]

Technical Field

[0002]

This invention relates to the field of molecular cell biology, specifically the application of CREPT, an essential protein for the formation of pluripotent stem cells, in induced pluripotent stem cells.

[0003]

Background Technology

[0004]

Stem cells are a type of pluripotent cell that can self-renew and differentiate into functional cells.

According to different classification standards, stem cells can be divided into different types. For example, based on the different developmental stages of stem cells, they can be divided into embryonic stem cells and adult stem cells; based on the differentiation potential of stem cells, they can be divided into totipotent stem cells, pluripotent stem cells, multipotent stem cells, and unipotent stem cells.

Embryonic stem cells are highly undifferentiated cells that can differentiate into various types of cells in the human body. They possess pluripotency and have brought convenience to medical transplantation treatment of diseases.

[0005]

However, ethical concerns and transplant rejection limit the medical applications of embryonic stem cells.

In 2006, Takahashi and Yamanaka successfully reprogrammed mouse fibroblasts into undifferentiated induced pluripotent stem (iPS) cells using four transcription factors (Oct4, Sox2, Klf4, and c-Myc, Yamanaka factor).

A year later, James Thomson's team successfully reprogrammed human somatic cells into pluripotent stem cells, or human iPS cells, using four factors: Oct4, Sox2, Nanog, and LIN28. The successful induction of iPS cells is of epoch-making significance. It means that the source of stem cells is no longer limited to embryonic stem cells or adult stem cells. Any type of cell in the human body can be induced into iPS cells for the treatment of diseases. Research on iPS cells has opened up new opportunities for life. The pluripotency of iPS cells endows them with extraordinary regenerative capabilities, which have immeasurable value in the medical field.

[0006]

The successful reprogramming of somatic cells further demonstrates that the maintenance of stem cell pluripotency is the result of multifactorial synergistic control.

These include transcription factors, chromatin remodeling enzymes, adaptor proteins, and chromatin structure regulatory proteins. Many tumor-related genes, such as c-Myc, Klf4, and STAT3, play important roles in maintaining the pluripotency of embryonic stem cells. Among them, c-Myc and Klf4 belong to the Yamanaka factor and are involved in the formation of iPS cells. CREPT (Cell-cycle Regulated and Expression-elevated Protein in Tumor) is a novel gene (GeneID: 58490) cloned and named by the applicant in recent years. It can promote tumor proliferation and metastasis and is a new tumor-related gene.

[0007]

Summary of the Invention

[0008]

The technical problem to be solved by this invention is how to prepare pluripotent stem cells and cell models that cannot be induced into pluripotent stem cells.

[0009]

To solve the above-mentioned technical problems, the present invention first provides the following method X1) or X2):

[0010]

X1) A method for preparing pluripotent stem cells, comprising: increasing the content and/or activity of CREPT protein in a starting animal cell named animal cell 1 to obtain a recombinant animal cell, and using the recombinant animal cell to prepare pluripotent stem cells; wherein the animal cell 1 is not a stem cell;

[0011]

X2) A method for preparing a cell model that cannot be induced into pluripotent stem cells, comprising: reducing the content and/or activity of the CREPT protein in a starting animal cell named animal cell 2 to obtain a cell model that cannot be induced into pluripotent stem cells; wherein the animal cell 2 is a non-stem cell and expresses the CREPT protein.

[0012]

Both animal cell 1 and animal cell 2 are isolated animal cells.

[0013]

The preparation of pluripotent stem cells using the recombinant animal cells can be carried out using the Yamanaka factor.

The Yamanaka factors are Oct4, Sox2, Klf4, and c-Myc.

[0014]

In the above method, the animal can be a1) or a2):

[0015]

a1) Mammals;

[0016]

a2) mice.

[0017]

In the above method, both animal cell 1 and animal cell 2 can be b1) or b2):

[0018]

b1) Somatic cells;

[0019]

b2) Fibroblasts.

[0020]

In the above method, the CREPT protein may be A1), A2), or A3):

[0021]

A1) The amino acid sequence is that of the protein in sequence 3;

[0022]

A2) Proteins with the same function but with one or more amino acid residues replaced and /or deleted and/or added, according to the amino acid sequence shown in Sequence 3 of the Sequence Listing.

[0023]

A3) is a fusion protein obtained by attaching a tag to the N-terminus and/or C-terminus of A1) or A2).

[0024]

To facilitate the purification of proteins in A1), tags as shown in the table below can be attached to the amino or carboxyl terminus of the protein, which consists of the amino acid sequence shown in Sequence 1 of the sequence listing.

[0025]

Table: Sequence of Labels

[0026]

Tag Residue Sequence Poly-Arg 5-6 (usually 5) RRRRR Poly-His 2-10 (usually 6) HHHHHH FLAG 8 DYKDDDDK Strep-tag II 8 WSHPQFEK c-myc 10 EQKLISEEDL HA 9 YPYDVPDYA

[0027]

The CREPT protein in A2) above is a protein that has 75% or more of the same amino acid sequence as the protein shown in Sequence 3 and has the same function.

The identity of 75% or more is defined as having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity.

[0028]

The gene encoding the CREPT protein in A2) above can be obtained by deleting one or more amino acid residues from the codons in the DNA sequence shown in Sequence 2, and/or by performing a missense mutation on one or more base pairs, and/or by attaching the coding sequence of the tag shown in the table above to its 5′ end and/or 3′ end.

The DNA molecule shown in sequence 2 encodes the CREPT protein shown in sequence 3.

[0029]

In the above method, the increase in the content and/or activity of CREPT protein in the starting animal cell named animal cell 1 can be achieved by introducing the gene encoding the CREPT protein into the animal cell 1.

[0030]

The reduction of the content and/or activity of the CREPT protein in the starting animal cell named Animal Cell 2 can be achieved by reducing or inhibiting the expression of the gene encoding the CREPT protein in Animal Cell 2.

[0031]

In the above method, the reduction or inhibition of the expression of the gene encoding the CREPT protein in the animal cell 2 can be achieved by knocking out the gene encoding the CREPT protein or any fragment thereof in the animal cell 2, or by introducing a substance that inhibits the gene encoding the CREPT protein into the animal cell 2.

[0032]

Specifically, reducing or inhibiting the expression of the gene encoding the CREPT protein in the animal cell 2 can be achieved by knocking out the exons of the gene encoding the CREPT protein in the animal cell 2.

The exon of the gene encoding the CREPT protein may be the third exon of the gene encoding the CREPT protein.

[0033]

The gene encoding the CREPT protein may be the DNA molecule shown in Sequence 2 of the sequence listing, and the genomic sequence of the gene encoding the CREPT protein is the DNA molecule shown in Sequence 1 of the sequence listing.

The sequence of exon 1 consists of positions 1-459, exon 2 (7500-7692), exon 3 (14910-15043), exon 4 (19423-19532), exon 5 (21591-21717), exon 6 (30156-30331), and exon 7 (46434-49711).

[0034]

In the above method, the knockout of the gene encoding the CREPT protein or any segment thereof can be achieved using the Cre-loxp method.

[0035]

The Cre-loxp method is a method that uses Cre recombinase and LoxP sites to inhibit the expression of target genes.

[0036]

The substance that inhibits the gene encoding the CREPT protein may be a nucleic acid molecule that reduces the expression level of the CREPT protein.

[0037]

The present invention also provides a cell model that cannot be induced into pluripotent stem cells prepared using the method for preparing the cell model that cannot be induced into pluripotent stem cells.

[0038]

This invention also provides the application of the CREPT protein or biomaterials associated with the CREPT protein in regulating the transformation of animal cells into pluripotent stem

cells; wherein the animal cells are not stem cells; and the biomaterials are any one of B1) to B9) below:

[0039]

B1) The nucleic acid molecule encoding the CREPT protein;

[0040]

B2) An expression cassette containing the nucleic acid molecule described in B1);

[0041]

B3) A recombinant vector containing the nucleic acid molecule described in B1), or a recombinant vector containing the expression cassette described in B2);

[0042]

B4) Recombinant microorganisms containing the nucleic acid molecules described in B1), or recombinant microorganisms containing the expression cassette described in B2), or recombinant microorganisms containing the recombinant vector described in B3);

[0043]

B5) A transgenic animal cell line containing the nucleic acid molecule described in B1), or a transgenic animal cell line containing the expression cassette described in B2);

[0044]

B6) Transgenic animal tissue containing the nucleic acid molecules described in B1), or transgenic animal tissue containing the expression cassette described in B2);

[0045]

B7) Transgenic animal organs containing the nucleic acid molecules described in B1), or transgenic animal organs containing the expression cassette described in B2);

[0046]

B8) Nucleic acid molecules that reduce the expression level of the CREPT protein;

[0047]

B9) Expression cassettes, recombinant vectors, recombinant microorganisms, transgenic animal cell lines, transgenic animal tissues, or transgenic animal organs containing the nucleic acid molecules described in B8).

[0048]

In the above applications, the nucleic acid molecule described in B1) may be as follows: b11), b12), b13), or b14):

[0049]

b11) The coding sequence is a cDNA molecule or DNA molecule of sequence 2 in the sequence listing;

[0050]

b12) The DNA molecule shown in sequence 1 of the sequence listing;

[0051]

b13) has 75% or more identity with the nucleotide sequence defined by b11) or b12) and is a cDNA molecule or DNA molecule encoding the CREPT protein;

[0052]

b14) hybridizes under stringent conditions to the nucleotide sequence defined by b11) or b12) or b13) and to a cDNA molecule or DNA molecule encoding the CREPT protein.

[0053]

The nucleic acid molecule can be DNA, such as cDNA, genomic DNA, or recombinant DNA; the nucleic acid molecule can also be RNA, such as mRNA or hnRNA.

[0054]

Those skilled in the art can readily mutate the nucleotide sequence encoding the CREPT protein of the present invention using known methods, such as directed evolution and point mutation.

Nucleotides that have been artificially modified and have 75% or higher identity with the nucleotide sequence of the CREPT protein isolated in this invention, as long as they encode the CREPT protein and have the function of the CREPT protein, are all derived from the nucleotide sequence of this invention and are equivalent to the sequence of this invention.

[0055]

The term "identity" used here refers to sequence similarity to natural nucleic acid sequences.

"Identity" includes nucleotide sequences that have 75% or higher, 85% or higher, 90% or higher, or 95% or higher identity with the nucleotide sequence of a protein composed of the amino acid sequence shown in the coding sequence 3 of the present invention.

Identity can be evaluated by the naked eye or by computer software.

Using computer software, the identity between two or more sequences can be expressed as a percentage (%), which can be used to evaluate the identity between related sequences.

[0056]

In the above applications, the stringent conditions can be as follows: hybridization at 50°C in a mixed solution of 7% sodium dodecyl sulfate (SDS), 0.5M NaPO-NER1, and 1mM EDTA, followed by rinsing at 50°C in 2×SSC and 0.1% SDS; hybridization at 50°C in a mixed solution of 7% SDS, 0.5M NaPO-NER2, and 1mM EDTA, followed by rinsing at 50°C in 1×SSC and 0.1% SDS; hybridization at 50°C in a mixed solution of 7% SDS, 0.5M NaPO-NER3, and 1mM EDTA, followed by rinsing at 50°C in 0.5×SSC and 0.1% SDS; hybridization at 50°C in a mixed solution of 7% SDS, 0.5M NaPO-NER4, and 1mM EDTA. Hybridization was performed in a mixed solution of EDTA, followed by rinsing at 50°C in 0.1×SSC and 0.1% SDS; alternatively, it could be performed at 50°C in 7% SDS, 0.5M NaPO4・NER5・, and 1mM EDTA・NER5・. Hybridization can be performed in a mixed EDTA solution, followed by rinsing at 65°C in 0.1

×SSC and 0.1% SDS. Alternatively, hybridization can be performed in a solution of 6×SSC and 0.5% SDS at 65°C, followed by rinsing once each with 2×SSC and 0.1% SDS and 1×SSC and 0.1% SDS. Alternatively, hybridization can be performed in a solution of 2×SSC and 0.1% SDS at 68°C, followed by rinsing twice for 5 min each time, then hybridization in a solution of 0.5×SSC and 0.1% SDS at 68°C, followed by rinsing twice for 15 min each time. Alternatively, hybridization can be performed in a solution of 0.1×SSPE (or 0.1×SSC) and 0.1% SDS at 65°C, followed by rinsing.

[0057]

The aforementioned 75% or higher degree of identity can be 80%, 85%, 90%, or 95% or higher degree of identity.

[0058]

In the above applications, the expression cassette (CREPT gene expression cassette) containing a nucleic acid molecule encoding the CREPT protein described in B2) refers to DNA capable of expressing the CREPT protein in a host cell. This DNA may include not only a promoter that initiates CREPT gene transcription but also a terminator that terminates CREPT gene transcription.

Furthermore, the expression box may also include an enhancing subsequence.

[0059]

Recombinant vectors containing the CREPT gene expression cassette can be constructed using existing expression vectors.

[0060]

In the above applications, the vector can be a plasmid, granule, bacteriophage, or viral vector.

[0061]

In the above applications, the microorganisms may be yeast, bacteria, algae, or fungi.

[0062]

In the above applications, the transgenic animal cell lines, transgenic animal tissues, and transgenic animal organs do not include reproductive materials.

[0063]

The animal may be a1) or a2):

[0064]

a1) Mammals;

[0065]

a2) mice.

[0066]

The animal cells may be b1) or b2):

[0067]

b1) Somatic cells;

[0068]

b2) Fibroblasts.

[0069]

In one embodiment of the present invention, the fibroblasts may be embryonic fibroblasts.

In another embodiment of the present invention, the fibroblasts are rat tail fibroblasts.

[0070]

This invention discovers that reducing the expression of CREPT in fibroblasts prevents fibroblasts from being induced to form iPS cells by four Yamanaka factors. However, expressing CREPT in cells that do not express CREPT can induce the cells to become iPS cells.

This indicates that CREPT and its encoding gene can be used to regulate the transformation of cells into iPS cells, to prepare cell models that cannot be induced into pluripotent stem cells, and to prepare iPS cells.

[0071]

Attached Figure Description

[0072]

Figure 1 shows a schematic diagram of the recombination of the recombinant DNA molecule as shown in sequence 4.

[0073]

Figure 2 shows the effect of CREPT gene knockout on the induction of MEF cells into iPS cells.

shCREPT represents the experimental group, and shNon represents the control group.

The left image shows the results of alkaline phosphatase staining.

***: P<0.01.

[0074]

Figure 3 shows the effect of CREPT gene knockout on the induction of rat tail fibroblasts into iPS cells.

OSKM represents the control group, OSKM+TAM represents the CREPT expression inhibition group, and OSKMC+TAM represents the CREPT expression restoration group.

***: P<0.01.

[0075]

Detailed Implementation

[0076]

The present invention will be further described in detail below with reference to specific embodiments. The embodiments given are only for illustrating the present invention and are not intended to limit the scope of the present invention.

Unless otherwise specified, the experimental methods described in the following examples are conventional methods.

Unless otherwise specified, all materials, reagents, instruments, etc. used in the following examples are commercially available.

In the quantitative experiments in the following examples, three replicate experiments were set up, and the average value of the results was taken.

In the following examples, unless otherwise specified, the first position of each nucleotide sequence in the sequence listing is the 5′ terminal nucleotide of the corresponding DNA, and the last position is the 3′ terminal nucleotide of the corresponding DNA.

[0077]

Example 1: Knockout of the CREPT gene inhibits the transformation of MEF cells into iPS cells.

[0078]

This embodiment found that the CREPT gene (Gene ID: 70470, NCBI (National Center for

Biotechnology Information)) plays an important role in maintaining the transformation of MEF cells into iPS cells.

The sequence of the CREPT gene is Sequence 1 in the sequence listing. Positions 1-459 of Sequence 1 represent the sequence of the first exon, positions 550-7499 the sequence of the first intron, positions 7500-7692 the sequence of the second exon, positions 7693-14909 the sequence of the second intron, positions 14910-15043 the sequence of the third exon, positions 15044-19422 the sequence of the third intron, positions 19423-19532 the sequence of the fourth exon, and so on. Positions 9533-21590 represent the sequence of the fourth intron, positions 21591-21717 represent the sequence of the fifth exon, positions 21718-30155 represent the sequence of the fifth intron, positions 30156-30331 represent the sequence of the sixth exon, positions 30332-46433 represent the sequence of the sixth intron, and positions 46434-49711 represent the sequence of the seventh exon. The CDS sequence of the CREPT gene is sequence 2 in the sequence listing, encoding the CREPT protein shown in sequence 3.

[0079]

I. Preparation of Materials

[0080]

1. Preparation of MEF cells

[0081]

MEF cells (Mouse Embryonic Fibroblast) were obtained from the embryos of mice that were 13.5 days pregnant (C57BL/6N, Beijing Vital River Laboratory Animal Technology Co., Ltd.), and were obtained after being expanded to the 3rd generation.

[0082]

2. Preparation of packaged CREPT shRNA virus

[0083]

According to AddGene's instructions, the shRNA sequence of CREPT was constructed into a lentiviral vector, with the shRNA target sequence being 5’-GGACCTGAGTTCACAAGAGA-3’, resulting in a recombinant lentiviral vector. This recombinant viral vector was then combined with packaging plasmids (AddGene, lentiviral vector pLL3.7 (http://www.addgene.org/11795/)); packaging plasmids psPAX2 (http://www.addgene.org/12260/) and pMD2.

G (http://www.addgene.org/12259/) was transfected into 293T cells, and the culture supernatant was collected 48 hours later, which is the viral supernatant for packaging CREPT shRNA.

The supernatant obtained by transfecting 293T cells with lentiviral vector and packaging plasmid was used as the control supernatant.

[0084]

3. Preparation of CREPT_NER6 mice

[0085]

The CREPT^{Flox/Flox} mice used were obtained from the MRC Harwell Institute in the UK. These mice were obtained by integrating the LoxP site into the flanking parts of the third exon of the CREPT gene in C57BL/6N mice using gene editing technology.

[0086]

The recombinant DNA molecule shown in Sequence 4 was electroporated into ES cells of C57BL6/N mice. The ES cell clones with correct recombination were obtained by G418 selection (i.e., the DNA fragment between positions 8948-19283 of the CREPT gene shown in Sequence 1 in the two chromosomes of C57BL6/N mice was replaced with the recombinant DNA molecule (the sequence of which is Sequence 4 in the sequence listing)). The recombination method is shown in Figure 1. In Figure 1, 1-6 represent the first to sixth exons of the CREPT gene, respectively.

Correctly recombined ES cell clones were injected into C57BL6/N mouse blastocysts to obtain chimeric mice. Then, chimeric mice were mated with C57BL6/N mice, and mice containing the recombinant fragment were selected as offspring, which are mice that can stably inherit the recombinant fragment. After self-crossing, homozygous mice with the recombinant fragment were obtained. Finally, these mice were mated with FRT tool mice (i.e., B6/JNju-H11em1Cin (CAG-FLPo)/Nju, preserved in the animal facility of Tsinghua University, from the Institute of Model Animals of Nanjing University, for details see http://www.nrcmm.cn/strain/2063) to obtain CREPT^{Flox}^{/Flox} mice.

[0087]

In sequence 4, positions 1-5472 and 13323-17292 are homologous arms, positions 5537-5584 and 12441-12488 are FRT sites, positions 10584-10617, 12495-12528 and 13266-13299 are LoxP sites, and positions 12945-13078 are the third exon of the CREPT gene.

[0088]

Ultimately, the CREPT^{Flox/Flox} mouse was obtained by replacing the DNA fragment between positions 8948 and 19283 of the CREPT gene shown in Sequence 1 of the two chromosomes of the C57BL6/N mouse with the recombinant DNA molecule shown in Sequence 5 of the sequence listing.

Sequence 5 contains homologous arms at positions 1-5472 and 6419-10388, FRT sites at

positions 5537-5584, LoxP sites at positions 5591-5624 and 6362-6395, and the third exon of the CREPT gene at positions 6041-6174.

[0089]

4. Preparation of mice with the CREPT^{Flox/Flox} and ERTII-Cre^{+/+} genotypes

[0090]

By comparing CREPT^{Flox/Flox} mice with EIIa-Cre tool mice (i.e., B6).

FVB-TgN(EIIa-Cre)C5379Lmgd,E2a-Cre, preserved in the animal facility of Tsinghua University, from The Jackson Laboratory, for details see https://www.jax.org/strain/003724) Mice with

the CREPT^{Flox/Flox} and ERTII-Cre^{+/+} genotypes were selected through mating and breeding identification.

[0091]

II. Effects of CREPT gene knockout on iPS cell formation

[0092]

1. Induction of iPS cells using MEF cells from C57BL/6J mice

[0093]

1) Cell counting: MEF cells were passaged into 6-well plates with gelatin-pretreated bottoms, with approximately 4000 cells added to each well. Culture medium (i.e., DMEM (Gibco, C11965500BT) with the following added: 10% (v/v) serum (Gibco, 10091-148) and 100 μM mercaptoethanol (SIGMA, M7522, all concentrations are in the culture medium) was added, and the cells were cultured at 37°C in a 5% CO2 NER16 cell culture incubator (SANYO). The volume of culture medium added to each well was 2 mL, with 3 parallel wells per group.

[0094]

2) After completing step 1) and culturing for 16 hours, add equal volumes of the viral supernatant of the four Yamanaka factors and the viral supernatant of the packaged CREPT shRNA from step 1 into a six-well plate and continue culturing, which is the experimental group.

The amount of Oct4, Sox2, Klf4 and c-Myc viral supernatant added to each well was 50 μL, and the amount of viral supernatant for packaging CREPT shRNA added was 100 μL.

The control supernatant from step one was used as a control group.

[0095]

The preparation methods for the viral supernatants of the four Yamanaka factors are as follows: The encoding gene of the Yamanaka factor was constructed into the lentiviral vector pLL3.7 according to the addgene instructions to obtain a recombinant lentiviral vector; the recombinant viral vector was then combined with the packaging plasmids psPAX2 and pMD2.

G was transfected into 293T cells, and the culture supernatant was collected 48 hours later, which is the Yamanaka factor virus supernatant.

[0096]

3) After completing step 2) and culturing for 24 hours, replace the medium with fresh medium. Thereafter, replace the medium every 24 hours. The fresh medium used for replacement is iPS cell induction medium. The iPS cell induction medium is obtained by adding the following substances to Knockout DMEM (Gibco, 10829-018): 20% (volume percentage) serum (Gibco, 10091-148), 2mM GlutMAX (L-alanyl-L-glutamine dipeptide, Gibco, 35050-061), 0.1mM NEAA (seven amino acids, Gibco, 11140-050, each amino acid has a concentration of 0.1mM in the medium), 100μM mercaptoethanol (SIGMA, M7522), and 10ng /mL LIF (Millipore, ESG1107). The concentrations of each substance are the concentrations in ES medium.

[0097]

4) After one week, iPS cells began to form in the control group, and after two weeks, iPS cell clones were clearly visible, while only a few iPS cells formed in the experimental group.

[0098]

5) Alkaline phosphatase (AP) staining: Alkaline phosphatase can specifically stain iPS cells but cannot stain fibroblasts.

The induced iPS cell clones were stained using the AP reagent kit.

Then, the six-well plates stained with AP were placed under an inverted microscope to observe the number of cell clones and take pictures.

[0099]

The results (Figure 2) showed that the percentage of iPS cell clones in the experimental group was 9±2% and the percentage of iPS cell clones in the control group was 100±9%. The percentage of iPS cell clones in the control group was significantly higher than that in the experimental group.

This indicates that decreased CREPT gene expression can inhibit the formation of iPS cells.

[0100]

2. Inducing iPS cells using tail fibroblast (TTF) cells from CREPT^{Flox/Flox};ERTII-Cre^{+/+} genotype mice.

[0101]

1) Ten weeks after birth, mice with the CREPT^{Flox/Flox};ERTII-Cre^{+/+} genotype were cut from the tail end (0.5 cm) to extract TTF.

[0102]

Cell counting: TTF counts of CREPT^{Flox/Flox};ERTII-Cre^{+/+} genotype mice were passaged into 6-well plates with gelatin-pretreated bottoms, with approximately 4000 cells per well. Culture medium (i.e., DMEM (Gibco, C11965500BT) with the following added: 10% (v/v) serum (Gibco, 10091-148) and 100 μM mercaptoethanol (SIGMA, M7522, all concentrations are in the culture medium) was added, and the cells were cultured in a 37°C, 5% CO2 cell culture incubator (SANYO). The volume of culture medium added to each well was 2 mL, with 3 parallel wells per group.

[0103]

2) After completing step 1) and culturing for 16 hours, equal volumes of the supernatant of the four Yamanaka factor viruses and TAM solution (a 5 mM solution of TAM (tamoxifen, SIGMA, catalog number T5648) dissolved in anhydrous ethanol) were added to a six-well plate, i.e., the CREPT expression inhibition group.

The amount of Oct4, Sox2, Klf4 and c-Myc virus supernatant added to each well was 50 μL, and the amount of TAM solution added was 2 μL.

[0104]

Following the above method, after completing step 1) and culturing for 16 hours, equal volumes of the supernatant of the four Yamanaka factor viruses, TAM solution (which is a 5 mM TAM solution obtained by dissolving TAM in anhydrous ethanol), and CREPT expression virus supernatant were added to a six-well plate to restore the CREPT expression group.

The amount of Oct4, Sox2, Klf4 and c-Myc viral supernatant added to each well was 50 μL, the amount of TAM solution added was 2 μL, and the amount of CREPT expression viral supernatant added was 100 μL.

[0105]

An experiment conducted simultaneously using an equal volume of anhydrous ethanol to replace the TAM solution served as a control group.

[0106]

3) After completing step 2) and culturing for 24 hours, replace with fresh culture medium. Replace the culture medium every 24 hours thereafter. All fresh culture medium used is iPS cell induction medium.

[0107]

4) After one week, iPS cells began to form in the control group, and after two weeks, iPS cell

clones were clearly visible. In the CREPT expression inhibition group, very few iPS cells formed, while in the CREPT expression restoration group, iPS cells began to form.

[0108]

5) Alkaline phosphatase (AP) staining: The induced iPS cell clones were stained using the AP kit.

Then, the six-well plates stained with AP were placed under an inverted microscope to observe the number of cell clones and take pictures.

[0109]

The results (Figure 3) showed that the percentage of iPS cell clones in the control group was 47±4%, the percentage of iPS cell clones in the CREPT expression inhibition group was

4±3%, and the percentage of iPS cell clones in the CREPT expression recovery group was 41±7%. The percentage of iPS cell clones in the control group and the CREPT expression recovery group was significantly higher than that in the CREPT expression inhibition group.

This indicates that inhibiting the expression of the CREPT gene prevents iPS cell formation, while re-expression of the CREPT gene restores iPS cell formation.

This indicates that the CREPT gene can regulate the formation of iPS cells.

[0110]

<110> Tsinghua University

[0111]

<120> Application of CREPT, an essential protein for pluripotent stem cell formation, in induced pluripotent stem cells.

[0112]

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[0113]

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