

A figure showing the analysis results of the RNA expression pattern in clone 6B7.

[Fig. 12]

Figures respectively showing A: analysis results of the urea synthesis amount of hepatocytes differentiated from clone 6B7, and B: analysis results of the angiogenic potential of vascular endothelial cells differentiated from clone 6B7.

[Fig. 13]

Figures showing the analysis results of the cell surface expression levels of PD-L1, PD-L2, HLA-G, and B2M in iPS cell clone 9G11 in which HLA class Ia & II are deleted and PD-L1, PD-L2, HLA-G, B2M, and iCasp9 genes are introduced by a flow cytometry method.

[Fig. 14]

Figures showing the analysis results of the cell surface expression levels of HLA-A, HLA-B, HLA-C, and HLA class II in clone 9G11 iPS cell-derived hematopoietic cells by a flow cytometry method.

[Fig. 15]

Figures showing the analysis results of the cell surface expression levels of PD-L1, PD-L2, HLA-G and B2M in clone 9G11 iPS cell-derived hematopoietic cells by a flow cytometry method.

[Fig. 16]

Figures showing the analysis results of the cell surface expression levels of undifferentiated markers in clone 9G11 by a flow cytometry method.

[Fig. 17]

A figure showing the results of the karyotype analysis in clone 9G11.

[Fig. 18]

Diagrams respectively showing A: analysis results of the urea synthesis amount of hepatocytes differentiated from clone 9G11, and B: analysis results of the angiogenic potential of vascular endothelial cells differentiated from clone 9G11.

[Fig. 19]

Figures showing proliferation rates of T cells (CD4-positive cells and CD8-positive cells) against CD45-positive cells differentiated from clone 9G11.

5 [Fig. 20]

A figure showing the cytotoxic activity of T cells (CD8-positive cells) against CD45-positive cells differentiated from clone 9G11.

[Fig. 21]

10 A figure showing the cytotoxic activity of NK cells against clone 9G11.

[Fig. 22]

A figure showing changes in cell viability after rapamycin treatment, in order to confirm the function of suicide gene of  
15 clone 9G11.

[Fig. 23]

Figures showing that the forced expression of exogenous B2M gene increases the expression level of HLA-G gene in HLA class Ia&II-deficient iPS cells.

20 [Description of Embodiments]

[0015]

The contents of the present invention are described in detail below.

[0016]

25 The present invention provides a hypoimmunogenic human cell having the following characteristics (1) to (5) (hereinafter the hypoimmunogenic human cell of the present invention):

- (1) lacking an endogenous gene encoding an  $\alpha$  chain of HLA class  
30 Ia,
- (2) lacking an endogenous gene encoding HLA class II or an expression regulator thereof,
- (3) comprising an exogenous gene encoding an  $\alpha$  chain of HLA class Ib,

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class Ia&II-deficient iPS cell clone 6B7 are shown below (Fig. 6).

#HLA-A: -13nt/+1nt

#HLA-B: -10nt/-17+1nt

#HLA-C: +1nt/-13nt

5 #RFXANK: -11nt/-10nt

[0123]

The following primers were used to amplify a DNA fragment containing the vicinity of the gRNA target sequence by a PCR method.

10 #RFXANK, Fwd: ATACCCACTCATGACGTGACCTG (SEQ ID NO: 11)

#RFXANK, Rev: CAGCCGCATCTCAAAGACAAG (SEQ ID NO: 12)

The length of the PCR product when using the above-mentioned primers is as follows.

#RFXANK: 410bp

15 [0124]

The cell surface expression level of HLA class Ia protein in HLA class Ia&II-deficient iPS cell clone 6B7 was analyzed using a flow cytometry method in the same manner as in Example 1. As a result, a drastic decrease in the cell surface expression level of HLA class I protein was confirmed in HLA class Ia&II-deficient iPS cell clone 6B7, like the cells obtained in Example 1 (Fig. 7).

[0125]

For the confirmation of the cell surface expression level of HLA class II protein in HLA class Ia&II-deficient iPS cell clone 6B7, the iPS cells need to be induced to differentiate into HLA class II-expressing cells. Therefore, hematopoietic cells including iPS cell-derived dendritic cell-like cells were utilized as such HLA class II-expressing cells.

30 Differentiation from iPS cells into hematopoietic progenitor cells followed the literature (Biochem Biophys Res Commun. 2019 Jul 12; 515(1):1-8.). In order to induce the differentiation of hematopoietic progenitor cells into hematopoietic cells including dendritic cell-like cells, iPS cell-derived  
35 hematopoietic progenitor cells were seeded on OP9 feeder cells,