様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

# 科学研究費助成事業　　研究成果報告書

令和　３ 年　 ６ 月 ２８ 日現在

機関番号：１４３０１
研究種目：研究活動スタート支援
研究期間：2019～2020
課題番号：１９Ｋ２３８６３

研究課題名（和文）Generation of universal donor cell souse for adoptive T cell therapy using iPSC technology

研究課題名（英文）Generation of universal donor cell souse for adoptive T cell therapy using iPSC technology

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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：同種T細胞を用いた免疫治療におけるHLA型不適合はレシピエント免疫細胞によるドナーT細胞拒絶の原因となる。我々は、HLA クラスIの構成分子であるB2M遺伝子、HLA クラスII転写因子のCIITA遺伝子、およびNK細胞活性化リガンドの一つであるPVRをノックアウトし、更にNK細胞の抑制性リガンドの一つである1本鎖HLA-Eを導入したiPS細胞とその由来のT細胞を作製した。また作成したiPS-T細胞は通常のiPS-T細胞よりも同種免疫細胞存在下、マウス生体内での生存能に優れており、それに基づく有意な抗腫瘍効果を示した。

研究成果の学術的意義や社会的意義
　ユニバーサルなT細胞免疫療法を目指してT細胞に直接ゲノム編集を行うさまざまな研究が行われてきましたが、ゲノム編行うことでT細胞の能力が弱まる可能性がある。本研究はこの問題を解決する方法として、iPS細胞の段階でゲノム編集を行い、それを分化させることでT細胞の弱体化を防ぎ、かつレシピエントの免疫細胞から攻撃を受けにくいT細胞を作製することに成功した。
　本研究で示した方法は、国内外で開発の進む他家iPS細胞を用いたT細胞免疫療法において、近い将来に応用される可能性があります。

研究成果の概要（英文）：Avoiding the immune rejection of transplanted T cells is central to the success of allogeneic cancer immunotherapies. One solution to protecting T-cell grafts from immune rejection involves the deletion of allogeneic factors and of factors that activate cytotoxic immune cells. Here we report the generation of hypoimmunogenic T cells derived from induced pluripotent stem cells (iPSCs) lacking β2-microglobulin, the class-II major histocompatibility complex (MHC) transactivator and the natural killer (NK) cell-ligand poliovirus receptor PVR, and expressing single-chain MHC class-I antigen E. In mouse models of CD20-expressing leukaemia or lymphoma, differentiated T cells expressing a CD20 chimeric antigen receptor largely escaped recognition by NKG2A+ and DNAM-1+ NK cells and by CD8 and CD4 T cells in the allogeneic recipients while maintaining anti-tumour potency. Hypoimmunogenic iPSC-derived T cells may contribute to the creation of off-the-shelf T cell immunotherapies.

研究分野：再生医療

キーワード：iPSC derived T cells　immune rejection　gene editing

## １．研究開始当初の背景

Autologous primary T cells engineered to express antigen-specific T cell receptors (TCRs) or chimeric antigen receptors (CARs) have great promise for clinical use against malignancies. However, the lengthy production process and high cost limit their large-scale clinical application. Using pre-banked allogenic T cells can circumvent these problems, but the immune rejection of transplanted allogenic T cells presents one major barrier. A recipient's CD8 T cells directly reject the grafted T cells by recognizing alloantigens (HLA class I or their presented antigens). Further, the recipient's CD4 T cells trigger the immune rejection of grafted T cells by recognizing other alloantigens (HLA class II or their presented antigens). Finally, the recipient's nature killer (NK) cells directly target grafted T cells that have a low expression of NK cell inhibitory ligands (normally HLA class I) and/or an overexpression of NK cell activating ligands. The regulation of these allogeneic factors and of factors that activate immune cells in T cells, mainly through gene modifications, should prevent immune rejection. However, multiple genetic modifications in primary T cells may lead to poor expansion and exhaustion.

We attempted to overcome these concerns by manipulating induced pluripotent stem cells (iPSCs), which can be maintained in an undifferentiated state before differentiation into mature T-cells. Gene modifications were performed in multiple steps in the clonal expandable iPSC stage. The iPSCs were then differentiated into T cells (iPS-T cells) and checked for immunogenicity.

In detail, we first checked that iPS-T cells naturally expressed HLA class I and upregulated HLA class II expression when activated. Accordingly, these iPS-T cells stimulated allogenic CD8 and CD4 T cells. To evade this stimulation, we generated HLA class I null iPSCs (B2MKO) by targeting the B2M gene, which encodes b2 microglobulin, a protein required for HLA class I presentation on the cell surface. We confirmed that B2MKO iPS-T cells do not induce the proliferation of allogenic CD8 T cells and escaped lysis. Next, we generated HLA class II null iPSCs (dKO) by knocking out the MHC class II transactivator CIITA in B2MKO iPSCs. Along with the B2MKO iPS-T cell effects on CD8 T cells, dKO iPS-T cells did not induce the activation or proliferation of allogenic CD4 T cells. However, HLA class I molecules also serve as major ligand inhibitors to NK cells. Indeed, we found that B2MKO and dKO iPS-T cells induced NK cell activation. NK cell activity is thought to be controlled by the balance of inhibitory and activating signals delivered via NK cell-surface receptors1. Conceivably then, increasing the level of ligands for inhibitory cell receptors on iPS-T cells or eliminating the ligands for NK cell activation receptors could abrogate the NK cell activation caused by B2MKO and dKO iPS-T cells. HLA-E (a ligand for the NK cell inhibitor receptor NKG2A) was reported to protect HLA class I down-regulated tissues, such as the placenta, and the overexpression of single chain HLA-E (B2M-HLA-E) protected B2MKO iPSC-derived CD45+ cells from NK cell lysis2. We therefore overexpressed HLA-E in dKO iPSCs (dKO/E iPSCs). dKO/E iPS-T cells caused low NKG2A+ NK cell activity. However, NKG2A- NK cells were still activated.

## ２．研究の目的

Further decrease the response by NK cells of the hypoimmunogenic dKO/E iPSC derived T cells and investigate their function and immunogenicity in humanized mouse model.

## ３．研究の方法

**Method to decrease the response by NK cells of the hypoimmunogenic (dKO/E) iPSC derived T cells**

Previous research has shown that activated primary T cells upregulate the expression level of NK cell-activating ligands for NKG2D35 and DNAM-134, resulting NK cells causing their death34,35. Accordingly, we screened the NKG2D ligands MICA, MICB and ULBP1–ULBP6 and the DNAM-1 ligands nectin-2 and PVR on activated and resting iPS-T cells. PVR was upregulated in activated iPS-T cells. Indeed, on activation, all iPS-T cells generated from B2MKO, dKO and dKO/E iPSCs showed much higher antigenicity for NK cells. On the basis of the evidence that human cytomegalovirus downregulates PVR on infected host cells to evade lysis by NK cells, we hypothesized that deleting PVR would enable iPS-T cells to evade the NK cell response independent of the HLA-E–NKG2A axis. PVR expression on iPSCs enabled us to rapidly generate PVR-knockout (tKO/E) iPSCs from dKO/E iPSCs via PVR-negative sorting after CRISPR–Cas9-mediated PVR knockout. The expression level of PVR in activated tKO/E iPS-T cells was similar

to that in the resting state.

**Method to investigate the function and immunogenicity of hypoimmunogenic iPSC derived T cells in humanized mouse mode.**

To evaluate the in vivo antitumor effects of tKO/E iPS-T cells for T cell immunotherapy in an allogeneic immune environment, we generated CD20-expressing B-LCLs from the PBMCs of a healthy donor as target cells and marked these cells with RLuc. In parallel, we transduced anti-CD20 CAR into WT and tKO/E iPS-T cells and marked both with FLuc. Single healthy donor derived PBMCs (1 × 106 cells) and B-LCLs (1×105 cells) were intraperitoneally injected into conventional NSG mice or into MHC-I- and MHC-II-knockout NOD/Shi-scid/Il2rgnull (NOG) mice (NOG/ MHC DKO). NOG/MHC DKO mice can maintain immune cells longer than NOG or NSG mice without severe acute xenobiotic GVHD. We then intraperitoneally injected the WT or tKO/E iPS-T cells in multiple cycles, while simultaneously monitoring the in vivo effector function and allogeneic immunogenicity.

## 4．研究成果

**NK cell activity of tKO/E iPSC derived T cells.**

On the basis of the expression of CD107a, a degranulation-related molecule, on the surface of co-cultured NK cells (CD3negCD56dim, bright), we found that tKO/E iPS-T cells decreased the activation of DNAM-1+ NK cells, which include NKG2A+ and NKG2A− NK cells, compared with parental PVR-intact dKO/E iPS-T cells

**Anti-tumor function and immunogenicity of tKO/E iPSC derived T cells i*n vivo* model.**

The injected tKO/E iPS-T cells immediately inhibited B-LCL growth, whereas the WT iPS-T cells needed about 12d to controlthe B-LCL growth in NOG/MHC dKO mice and in NSG mice. In addition, tKO/E iPS-T cells were more stable than WT iPS-T cells after every injection in both mouse types. These observations could be explained by the poor rejection of tKO/E cells from the mouse body by alloreactive human PBMCs. As for the suppression of B-LCLs after 12d, this effect is in part due to the expansion of EBV antigen-specific CTLs from the original PBMCs.

Since the clearance of WT iPS-T cells became faster, especially from the second round of injections, we speculated that immunological memory against WT iPS-T cells had formed. To clarify how many alloreactive immune cells existed in vivo, we isolated healthy donor-derived immune cells from the spleens of NSG mice and analyzed them by flow cytometry. Isolated residual human CD45+ cells from the spleen (Supplementary Fig. 6f) of a WT iPS-T-treated NSG mouse were co-cultured with irradiated WT iPS-T cells or tKO/E iPS-T cells to measure reactive T cells. WT iPS-T cells induced a much higher proliferation of donor-derived CD4 and CD8 T cells, suggesting immunological memory formation in vivo, whereas tKO/E cells did not . In the case of non-xenoreactive NOG/MHC dKO mice, significantly more alloreactive human PBMCs were observed only in WT iPS-T injected mice. This observation confirmed that the multicycle challenge of WT iPS-T cells induced a systemic immunological memory effect, but that tKO/E iPS-T cells did not induce a statistically significant allogeneic response. Collectively, we showed that tKO/E iPS-T cells survived and exerted effector functions to inhibit tumor progression in an allogeneic immune-cell grafted mouse mode

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　1件）

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| １．著者名<br>Bo Wang, Shoichi Iriguchi, Masazumi Waseda, Norihiro Ueda, Tatsuki Ueda, Huaigeng Xu, Atsutaka Minagawa, Akihiro Ishikawa, Hisashi Yano, Tomoko Ishi, Ryoji Ito, Motohito Goto, Riichi Takahashi, Yasushi Uemura, Akitsu Hotta and Shin Kaneko | ４．巻<br>5 |
| ２．論文標題<br>Generation of hypoimmunogenic T cells from genetically engineered allogeneic human induced pluripotent stem cells | ５．発行年<br>2021年 |
| ３．雑誌名<br>Nature Biomedical Engineering | ６．最初と最後の頁<br>429-440 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.1038/s41551-021-00730-z | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスとしている（また、その予定である） | 国際共著<br>該当する |

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　1件）

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| １．発表者名<br>Bo Wang（王　博） |
| ２．発表標題<br>Generation of "universal iPSC" with enhanced stealth ty on iPSC for allogeneic T cell immunotherapy |
| ３．学会等名<br>第11回日本血液疾患免疫療法学会学術集会 |
| ４．発表年<br>2019年 |

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| ２．発表標題<br>Enhanced stealth property on iPSC to generate universal T cell source for allogeneic cancer immunotherapy |
| ３．学会等名<br>17th International Congress of Immunology（国際学会） |
| ４．発表年<br>2019年 |

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| １．発表者名<br>Bo Wang（王　博） |
| ２．発表標題<br>Universal T cell generation for off-the-shelf cancer immunotherapy from gene edited allogeneic iPSCs |
| ３．学会等名<br>第19回日本再生医療学会総会 |
| ４．発表年<br>2020年 |

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

| | 氏名<br>（ローマ字氏名）<br>（研究者番号） | 所属研究機関・部局・職<br>（機関番号） | 備考 |
|---|---|---|---|

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

| 共同研究相手国 | 相手方研究機関 |
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