主論文の要旨

No Immunogenicity of IPS Cells in Syngeneic Host Studied by *In Vivo* Injection and 3D Scaffold Experiments

〔 IPS 細胞は自己に移植した場合免疫原性を持たない 〕

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Introduction

Induced Pluripotent Stem Cells (iPSCs), one of the greatest inventions of the 21st century are nothing but embryonic stem cell like cells generated from somatic cells by the introduction of defined transcription factors Oct3/4, Sox2, Klf4, and c-Myc. Since iPSCs possess the ability to differentiate into various kinds of cells, successful differentiation of iPSCs to various somatic cells pertaining to different germ layers like neurons, cardiovascular progenitor cells, hepatocytes, and so forth is being reported repeatedly in recent years. However the survival of transplanted iPSCs *in vivo* is questioned in a recent paper by Zhao et al. stating that transplantation of iPSCs derived from Mouse Embryonic Fibroblasts by retroviral reprogramming evokes an acute immune response in syngeneic recipients. To treat damaged tissues or dysfunctional organs in elderly patients, it is necessary to establish iPSCs from the patient's own tissue. We succeeded in establishing iPS clones (aged iPSCs) using bone marrow (BM) of 21-month-old EGFPC57BL/6 (EGFP-C57BL/6) mice that had been cultured for four days in granulocyte macrophage-colony stimulating factor (GM-CSF). In this study we analyzed the immunogencity of iPSCs clones derived from different ages of mice.

Method

We determined the immunogenicity of three different iPSC clones, MEF iPSCs, 15 month aged iPSCs and 21 month aged iPSCs by their ability to form teratoma upon subcutaneous injection. The ultra structures of the teratoma were studied by Transmission Electron Microscopy (TEM). MEF iPSCs and myeloid differentiated MEF iPSCs were cultured in 3D scaffolds followed by transplantation of these scaffolds in C57BL/6 mice and BALB/c mice. The cell proliferation rate of iPSCs and the T cell infiltration inside the scaffolds transplanted into syngeneic and allogeneic mice were compared by histological staining.

Results

In this study we analyzed the immunogenicity of iPSCs clones derived from different ages of mice by retrovial reprogramming. MEF iPSCs, aged-iPSCs derived from 21-month-old male were established previously. We added newly established iPSCs clones (clone 1 and clone 2) derived from 15 months old C57BL/6 mice. We investigated the teratoma formation potential of different iPSCs clones by subcutaneous injection of the cells in C57BL/6 and BALB/c mice. We found that the injection of two clones of 21-month old iPSCs and two clones of 15-month-old iPSCs resulted in teratoma after 3 weeks, whereas the teratoma forming ability in MEF iPSCs is 92.86%. There was no teratoma formation by any of the iPSCs in allogeneic BALB/c mice (Table 1). By performing Transmission Electron Microscopy (TEM), we confirmed the presence of different cell types pertaining to ectoderm, endoderm, and mesoderm in teratoma of MEF-derived iPSCs (Figure 1).

In order to evaluate immune cell infiltration into teratoma made by the transplantation of iPSCs either into C57BL/6 or BALB/c mice, we made use of three-dimensional (3D) porous scaffold made by PLGA (poly D, L- Lactic acid glycolic acid) for the *in vitro* culture and transplantation of iPSCs subcutaneously into the host. MEF iPSCs were seeded in the scaffold, cultured for 2 weeks and transplanted into C57BL/6 and BALB/c mice. H& E staining of the explanted scaffolds at different time periods shows that the cell density inside the scaffolds transplanted to syngeneic C57BL/6 mice increases rapidly, whereas the scaffolds transplanted to allogeneic BALB/c mice had low cell density (Figure 2). Neither in BALB/c nor in C57BL/6 mice could we observe CD4 or CD8 T cell infiltration at day 6 after transplantation. However at day 20 there is increase in the number of CD8 T cells in allogeneic host but we could not observe a CD4 positive cell in any host type. We also examined secondary challenge of MEF iPSCs in scaffold. We first injected a total of 2×10^6 cells of splenocytes from C57BL/6 mouse into BALB/c or C57BL/6 mice as a primary challenge followed by transplantation of iPSCs cultured scaffold after 2 weeks. At day 4 and day 8, we could observe immediate increase in CD8 as well as CD4 T cell number in the allogeneic explants. In contrast, we could not detect any CD8 or CD4 positive T cells at day 4 and only a few CD4 cells could be detected at day 8 in syngeneic explants. These results indicate that neither in primary challenge nor in secondary challenge T cell infiltration was induced by MEF iPSCs upon syngeneic transplantation (Figure 3).

We next explored the immunogenicity of completely differentiated iPSCs by culturing in 3D scaffolds. We made use of myeloid differentiated C57BL/6-derived MEF iPSCs, which were previously established. We found that the cell proliferation rate was relatively slow and not significantly different in syngeneic and allogeneic hosts up to 6 days after transplantation. However after 20 days, the cell density in syngeneic explants was found to be 70% greater than the allogeneic one. In the case of evaluating T cell infiltration, neither CD4 nor CD8 positive cells could be observed until 6 days after transplantation of the scaffolds in any of the recipients. But, after 20 days about 87% increase in infiltrating CD8 positive cells were observed in scaffolds from allogeneic mice compared to their syngeneic counterpart. However the number of CD4 positive cells that could be detected is highly negligible in both types (Figure 4).

Discussion

Zhao et al. have shown that iPSCs made by retroviral reprogramming are immunogenic, showing that teratoma developed by those iPSCs were rejected by syngeneic mice by inducing T cell infiltration. Their results are contradictive to our results. One possibility of differences between our results and the results of Zhao et al. is that during the course of iPSCs production some changes undergone by iPSCs like copy number variation, infrequent DNA rearrangement, chromosomal aberrations etc., could induce neoantigens which will be recognized by syngeneic host. These changes may be included in some cells in one clone, which are derived from fibroblasts in skin. In our experiments, only one in 14 trials of teratoma transplantation derived

from MEF iPSCs was rejected whereas, all clones derived from bone marrow were not rejected by syngeneic host.

For clinical application, immunogenicity of differentiated cells must be examined. We have reported model transplantation works of differentiated iPSCs into syngeneic mice and have shown that transplanted cells have been detected *in vivo*. Although we could detect differentiated iPSCs *in vivo*, the number of detected iPSCs was few. First possibility is that transplanted iPSCs might be dead due to their low ability to grow *in vivo* and phagocytosed by macrophages. Second possibility is that transplanted iPSCs are diffused *in vivo*, thus it is difficult to detect by tissue staining. Third possibility is that differentiated iPSCs expressed altered-self, which might be rejected by immune T cells. Here we clearly showed that differentiated macrophages were not rejected by T cells of syngeneic mice by using scaffold transplantation. During the course of preparing manuscript, Araki et al. reported that terminally differentiated cells derived from iPSCs are not immunogenic by showing that differentiated iPSCs-derived skin or bone marrow cells transplanted to syngeneic host were not rejected by T cells. Considering these results and our results presented here, iPSCs have no immunogenicity in syngeneic recipient with or without differentiated form.

Conclusion

Here we show that iPSCs are not rejected in syngeneic host. Nonimmunogenicity of iPSCs is not restricted to MEF derived iPSCs. Aged-iPSCs are also not rejected in syngeneic mice. These findings favor the possibility to use iPSCs from patient own tissue to treat incurable diseases such as liver, lung and kidney failure.