Immunogenicity of Induced Pluripotent Stem Cells

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A new study shows the immunogenicity of induced pluripotent stem cells by the direct transplantation of undifferentiated cells into syngenic mice.

The reprogramming of somatic cells into pluripotent stem cells has been reported after introducing a combination of several defined factors, such as OCT3/4, SOX2, KLF4, and c-MYC, into the cells.[1] These artificially established cells are termed induced pluripotent stem cells (iPSCs). The iPSCs show unlimited growth while maintaining their potential for differentiation into various cell types of all 3 germ layers. Their pluripotency has been clearly shown by their contribution to chimeric animals and by the development of a full-term mouse during a tetraploid complementation experiment. These data also indicated that cells differentiated from iPSCs can at least partially replace the biological functions of various organs. Unlike embryonic stem cells (ESCs), iPSCs can be generated from a patient's own somatic cells. Therefore, the potential utility of iPSCs for regenerative medicine has been suggested.

The development of iPSC-derived differentiated cells has been expected to provide personalized cells for cell-based therapy. However, the immunogenicity of these cells had not yet been strictly examined. Recently, Zhao et al. reported that the transplantation of immature iPSCs induced a T-cell–dependent immune response even in a syngenic mouse.[2] When injected into immunodeficient mice, undifferentiated pluripotent cells grow locally, differentiate, and form teratomas that contain various cell types, including neurons, cartilage, keratinocytes, and intestinal epithelium. In this study, the authors investigated the immunoreactions to iPSCs and ESCs in immunocompetent mice using 3 types of experimental models: an iPSC autograft, an ESC autograft, and an ESC allograft (Table).

The syngenic transplantation of C57BL/6 (B6)-derived ESCs into B6 mice showed a high frequency of teratoma formation (97%), whereas 129/SvJ-derived non-syngenic ESCs did not develop teratomas except in 1 mouse, in which they found vigorous T-cell infiltration, a landmark of immune system rejection. On the other hand, syngenic iPSCs established by retroviral vectors (ViPSCs) generated teratomas in 65% of the transplantation sites after 30 days. All the ViPSC-derived teratomas they examined showed T-cell infiltration. The retroviral vectors integrated the reprogramming factors into the genome of the ViPSCs, and the authors found that the teratomas expressed Oct3/4 from their integrated loci. A recent report revealed the existence of T-cells that naturally target Oct3/4 proteins in peripheral blood.[3] Therefore, the authors suggested that the high T-cell infiltration of the ViPSC-teratomas was due to the ectopic expression of Oct3/4.

To avoid this issue, they used 3 lines of non-integrated episomally-derived iPSCs (EiPSCs) for further experiments. The tumor formation rate of these EiPSCs was 84%. However, more than half of them still showed T-cell infiltration and some tumors regressed during the observation period. The authors subsequently observed that 9 genes were commonly expressed in the regressed teratomas derived from 2 lines of EiPSCs and demonstrated that 3 of these genes, Zg6, Hormad1, and Cyp3a11, interfered with the teratoma formation of syngenic ESCs when overexpressed. Hormad1 had previously been identified as a tumor antigen[4] and was also expressed in most teratomas developed from integration-free iPSCs, which were independently induced using different methods involving adenoviral vectors, plasmids, and recombinant proteins. Finally, the authors used $CD4^{-/-}$ or $CD8^{-/-}$ mice to confirm that the interference with tumor formation was due to the T-cell activity. As expected, the robust immune reaction of ViPSCs and the ESCs expressing Hormad1 was abolished. In addition, no regression was observed in the teratomas formed by EiPSCs. Therefore, both $CD4^{+}$ helper T-cells and $CD8^{+}$ cytotoxic T-cells are essential for the observed immune reactions.

This is a very important study; however, a more detailed investigation is needed to confirm their results. First of all, the authors compared only 1 line of syngenic ESCs with several lines of iPSCs. ESCs themselves have been shown to have a wide range of diversity in their differentiation potential.[5] A study using additional ESC lines would be necessary to prove that the immunogenicity observed in this article is specific for iPSCs.

In addition, they used undifferentiated iPSCs for transplantation, which would never be used for medical applications. When tumorigenic cells emerge in the body, the immune system can recognize and try to eliminate them. Hence, it is not surprising that there was infiltration of T-cells into the developing teratomas. The results of the study by Zhao et al. might simply reflect the immunoreactions against undifferentiated cell transplantation. In that case, the iPSCs they used would likely have differentiated more slowly than the control ESCs. There have already been several reports describing the

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Table. Summary of Teratoma Formation on Day 30

Type of Model	Donor Cell	Recipient	Average Teratoma Formation Rate (%)	Lymphocytic Infiltration
iPSC autograft	ViPSC (B6)	Mouse (B6)	64.7% (22/34)	+ (10/10)
iPSC autograft	EiPSC (B6)	Mouse (B6)	83.9% (26/31)	+ (8/13), − (5/13)
ESC autograft	ESC (B6)	Mouse (B6)	96.8% (30/31)	−
ESC allograft	ESC (129/SvJ)	Mouse (B6)	3.2% (1*/31)	+ (1/1)

The average tumor formation rates for iPSC autograft and ESC allograft/autograft models. This table was calculated based on Figures 1B and 3D, and Supplemental Figure 3E in the article.[2] The authors noted that only small teratoma formation was observed in the ESC allograft model (asterisk).

amelioration of the symptoms of rodent disease models after transplantation of iPSC-derived differentiated cells. For example, hematopoietic stem cells and neuronal precursor cells from iPSCs could relieve sickle cell anemia and spinal cord injury, respectively.[6,7] The immunogenicity of the differentiated cells should be assessed. Moreover, it is also essential to examine the expression of Hormad1 in these other types of differentiated cells. The mechanisms underlying the control of Hormad1 expression could be helpful to understand the reprogramming process and improve iPSC induction methods.

When considering the human medical setting, autologous iPSCs, but not ESCs, can be established from each patient. Therefore, it would be more meaningful to compare transplantation of autologous iPSCs with allologous and MHC-matched ESCs instead of autologous ESCs. From more practical point of view, however, the autologous derivation of iPSCs would require a lot of time for assessment of their medical stability, safety, and efficacy. Therefore, several scientists have proposed the establishment of an MHC-typed bank of pluripotent stem cells for cell transplantation therapy.[8] Therefore, it would be important to analyze MHC-matched allologous iPSCs and ESCs under the treatment of low-dose immunosuppression. Several studies have suggested that there are differences between ESCs and iPSCs in terms of their gene expression profile, epigenetics, and differentiation potential. Both ESCs and iPSCs would develop mutations in their genome during the cultivation process. In addition, iPSCs would have extra mutations, as they are reprogrammed from aged somatic cells. Therefore iPSCs and ESCs could be expected to have abnormally expressed gene(s) after differentiation, which might evoke an immune response. However, if the immunogenicity of such cells is weak, we will be able to control the immune responses of the recipient through immunosuppressant treatment. It is therefore necessary to perform careful and practical examinations for these kinds of assays and to consider how much risk is acceptable for cell transplantation therapy.

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Disclosures

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