Mini Review

Immune cells and iPS cells

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Cell reprogramming technology opens a new insight in not only basic biology but also clinical medicine including immune cell therapy. Application of cell reprogramming technique in manipulating immune cells may bring beneficial results in tumor immunotherapy. In this manuscript, current status of the cell reprogramming in immune cells especially lymphocytes is summarized, and its perspective is discussed.

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Innovative technology to generate pluripotent stem cells from differentiated somatic cells by transducing only a few factors, i.e., induced pluripotent stem (iPS) cells, has been recently established[1-3]. iPS cells are expected to be used for not only basic research but also regenerative medicine and development of new drugs.

Reprogramming of immune cells to pluripotent stem cells

Firstly, attempts to reprogram some differentiated somatic cells to induce pluripotent stem cells were successfully performed by using fibroblast cells[1]. After the initial successful experiments, conversion of various kinds of somatic cells such as liver and stomach cells into pluripotent stem cells has been reported[4]. However, at that time, it was still unclear whether terminally differentiated cells had a potential to reprogram into a pluripotent state. There was a possibility that only the cells closer to stem cell state accepted the reprogramming to pluripotent state. B and T cells have a favorable feature to examine the reprogramming feasibility of terminally differentiated cells. In B and T cells, sequential intrinsic genetic DNA rearrangement in their antigen receptor gene loci genetically mark the different consecutive stages of their maturation. In 2008, Hanna et al. successfully demonstrated that generation of mouse iPS cells from terminally differentiated B cell receptor gene-rearranged B cells[5].

In their observation, 4 Yamanaka factors (*Oct4, Sox2, Klf4* and *c-Myc*) were not sufficient to convert bone marrow Igκ^+ mature B cells and spleen IgM$^+$ IgD$^+$ mature B cells to pluripotent state. It was required ectopic expression of *C/EBP*α or specific knockdown of *Pax5* to reprogram the mature B cells. However, in our recent research, mouse splenic CD19$^+$ B cells could be converted into pluripotent state with only 4 Yamanaka factors[6].

Recently, some groups reported generation of human iPS cells

from peripheral blood[7-9]. Seki et al.[7] successfully generated iPS cells from circulating T cells at 0.1% of efficiency. They used only 1 ml of blood samples, and introduced the four factors (*OCT4, SOX2, KLF4* and *c-MYC*) into activated T cells using Sendai-virus vectors. The iPS cell derivation system they developed has advantages in addition to the relatively high efficiency. Gene expression system using the Sendai-virus vectors can induce integration-free iPS cells. It is important to leave the genome intact for avoiding development of cancer and also for normal differentiation of iPS cells.

Development of immune cells from iPS cells

Differentiation of iPS cells into immune cells is being considered for establishing new therapeutic tools for treating some haematological or immunological disorders.

Senju et al. reported that mouse embryonic fibroblast derived iPS cells can differentiate into functional dendritic cells and macrophages[10]. Lei et al. reported that mouse iPS cells can differentiate into T cells[11]. However, information regarding differentiation into B cells from iPS cells has been limited.

Establishment of induced pluripotent stem cell from peripheral B cells and its differentiation into T-cell or B-cell lineage

It has previously been demonstrated that haematopoietic stem and ES cells can differentiate into both T and B cells in a coculture system using OP9 cells as feeder[12]. We were able to induce differentiation of mouse embryonic fibroblast-derived iPS (MEF-iPS) cell into T-cell lineage, including $CD4^+CD8^+$ cells, $CD3^+CD8^+$ cells, $\alpha\beta$ TCR^+ T cells and $\gamma\delta$ TCR^+ T cells, using Delta like-1-expressing-OP9 co-culture system. We also found that IFN-γ production by T-cell receptor stimulation in the T cells derived from MEF-iPS cells.

On the other hand, it was relatively difficult to induce B-cell lineage differentiation from MEF-iPS cells in the OP9-co-culture system which has been reported to be supportive environment for B-cell lineage differentiation. We hypothesised that this disparity was, at least in part, dependent on the source of iPS cells. Therefore, we attempted to generate iPS cells from B cells, using the 'classical' retroviral transduction of 4 Yamanaka transcription factors (*Oct4, Sox2, Klf4*, and *c-Myc*)[1]. We successfully generated iPS cells from murine splenic $CD19^+$ B-cells (B-iPS), from which we could generate teratoma containing cell types representing all three embryonic germ layers *in vivo*. *In vitro*, B-iPS cells efficiently differentiated into T-cell lineage, in contrast, were resistant to differentiate into B-cell lineage similar to that of MEF-iPS cells. We further analyzed the expression of *Ebf1* and *Pax5*. Those transcription factors are known to critical for B-cell lineage development. In hematopoietic progenitor cells from iPS cells, *Ebf1* transcript but not *Pax5* was detected. However, CpG methylation status of *Pax5* promoter region in MEF-iPS and B-iPS cells were largely unmethylated as in splenic $CD19^+$ cells. It suggests that the failure of *Pax5* expression in iPS cells was not caused by the epigenetic modification of the *Pax5* gene. We attempted that enforced expression of *Pax5* rescues the B cell development, but retroviral forced expression of *Pax5* was ineffective for the B-cell lineage development from MEF-iPS cells and B-iPS cells. Importantly, *Oct4* transcripts from retrovirally transduced ones were detected in MEF-iPS and B-iPS cells-derived hematopoietic progenitors. The ectopic *Oct4* expression seen in the cells differentiated from iPS cells may regulate the *Pax5* expression and also B-cell lineage differentiation. Kim et al. reported that low-passage iPS cells derived from adult murine tissues retain residual DNA methylation signatures characteristic of their somatic tissue of origin[13]. The "footprint" epigenetic signature may favour their differentiation along lineages related to the donor cell. According to their observation, it is seemingly that iPS cells from peripheral B cells favour to differentiate into B-cell lineage, but it was not the case. Recently, Szabo et al. reported that only enforced expression of *Oct4* in dermal fibroblast cells directly derivate $CD45^+$ hematopoietic progenitor cells. They successfully gave rise to granulocytic, monocytic, megakaryocytic and erythroid lineages, and demonstrated *in vivo* engraftment capacity. However, they did not detect lymphoid cell differentiation from the hematopoietic progenitor cells generated by Oct4 transduction into fibroblast[14]. In these contexts, *Oct4* expression in hematopoietic progenitor cells may actively interfere the B-cell lineage development.

Derivation of iPS cells from antigen specific immune cells and generation of antigen specific immune cells

In the manipulation to induce iPS cells, although epigenetic alteration could occur, gene sequences should not be altered. Therefore, this feature of cell reprogramming technology could be useful for researches of various inherited disease. This feature is also beneficial for immunological treatment using iPS cells. In the development of T and B cells, rearrangement of the antigen receptor genes makes diversity of antigen specificity of the cells. However, the antigen-receptor gene rearrangement is random and we can not control the rearrangement. The prob-

ability to obtain a particular cell which harbor beneficial antigen-receptor what we need is quite low.

NKT cells are a kind of T cells, characterized by their distinctive T cell receptor alpha chain encoded by Vα14-Jα18 in mice and by Vα24-Jα18 in humans, and their recognition of glycolipid antigens in the context of CD1d. NKT cells are thought to be useful for anti-tumor immunotherapy. However, most tumor-bearing patients do not have sufficient numbers of NKT cells.

When we generate iPS cells from a lymphocyte which has an useful antigen-receptor, the iPS cells give rise to the lymphocytes which harbor the same "useful" antigen-receptor. This idea has been already guaranteed in cloned mice from NKT cells by somatic cell nuclear transfer[15]. In these mice, NKT cells comprised the most population of T cells.

In a recent research, Taniguchi and his colleagues generated iPS cells from NKT cells, and they also showed a differentiation into functional NKT cells from the NKT cell-derived iPS cells *in vitro*[16]. This study represents one of the instance of the immune cell reprogramming. This result would be applicable for other T cells, such as tumor specific cytotoxic T cells or B cells.

To preserve the iPS cells which can produce useful immune cells such as tumor specific T cells would help establishing a new immunotherapy. Recent research revealed that only single gene, *Oct4* transduction into human dermal fibroblast cells directly convert to multi-lineage blood progenitors without establishing pluripotent state. However, lymphoid differentiation capacity was defect in their observation. Thus, it seems that there still exist some problems to be solved in reprogramming or differentiating immune cells. In the near future, further studies may resolve these issues, and a development of new immunotherapy collaborated with the cell reprogramming technology is expected.

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