Establishment of immortalized human erythroid progenitor cell lines able to produce enucleated red blood cells

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Background  In many countries, the available supply of transfusable red blood cells (RBCs) is often inadequate. This problem has stimulated considerable research around the world into the development of a robust method for in vitro production of transfusable RBCs. Some possible methods are under active investigation such as the production of RBCs from hematopoietic stem/progenitor cells and from embryonic stem (ES) or induced Pluripotent Stem (iPS) cells. Although it may be feasible to produce RBCs from their immediate precursor cells, it is not easy to obtain sufficient numbers of these precursor cells. One solution to the latter problem may be to establish immortalized erythroid progenitor cell lines able to produce transfusable RBCs in vitro; these immortalized cells would form a very valuable resource. We previously developed a robust method for generating immortalized erythroid progenitor cell lines following the induction of hematopoietic differentiation of mouse ES cells, and successfully established several immortalized erythroid progenitor cell lines that we designated Mouse ES cell Derived-Erythroid Progenitor (MEDEP) cell lines. Although the precise characteristics of these cell lines varied, each of them could differentiate in vitro into more mature erythroid cells, including enucleated RBCs. Transplantation of MEDEP cells into mice suffering from acute anemia resulted in transient proliferation of the cells, which subsequently differentiated into functional RBCs and significantly ameliorated the acute anemia.

Aims  Establishment of immortalized human erythroid progenitor cell lines able to produce enucleated RBCs.

Methods  We sought to establish immortalized erythroid progenitor cell lines following the induction of hematopoietic differentiation of human ES cells and iPS cells using a similar method as for establishment of MEDEP cell lines (Hiroyama et al., PLoS ONE 3: e1544, 2008).

Results  Using the original method developed in mice, we failed to establish immortalized human erythroid progenitor cell lines. This may have been due to the low efficiency of inducing erythroid cells from ES and iPS cells. It was previously reported that enforced expression of the transcription factor TAL1, which plays essential roles in early hematopoiesis and differentiation of erythroid cells and megakaryocytes, improved the efficiency of induction of hematopoietic cells from ES cells of the common marmoset. We therefore enforced expression of TAL1 in human iPS cells and found that this resulted in a significant improvement in the rate of production of hematopoietic cells, in particular of erythroid cells. However, our attempts to establish immortalized human erythroid progenitor cell lines...
from iPS cells expressing Tal1 were unsuccessful. Recently, it was reported that enforced expression of HPV16-E6/E7 in CD36-positive erythroid cells aided the establishment of immortalized human erythroid progenitor cell lines. We decided to adopt this strategy with some modifications and succeeded in establishing immortalized human erythroid progenitor cell lines able to produce enucleated RBCs.

Summary/Conclusions We have successfully established immortalized Human Umbilical Cord Blood Derived-Erythroid Progenitor (HUDEP) cell lines and Human iPS cell Derived-Erythroid Progenitor (HiDEP) cell lines that are able to produce enucleated RBCs.

Key words: blood transfusion, cell lines, erythropoiesis, ES cells, iPS cells, red blood cells.

Introduction

The availability of transfusable red blood cells (RBCs) is inadequate in many countries. This problem has stimulated a considerable amount of research around the world into establishing robust methods for the in vitro production of transfusable RBCs. There are various possible routes for production of RBCs, such as using hematopoietic stem/progenitor cells, embryonic stem (ES) cells, or induced Pluripotent Stem (iPS) cells.

Immortalized cell lines, such as ES cells and iPS cells are particularly attractive and promising resources for in vitro production of RBCs as they can proliferate without limitation. However, it should be noted that the efficiency of generation of erythroid progenitor cells and RBCs varies with the culture methods employed and the ES cell lines used in the system. Even with the optimal experimental procedure and the most appropriate ES cell line, the generation of abundant RBCs directly from primate ES cells is a time-consuming process [1]. An alternative approach is to produce RBCs from their immediate precursor cells; however, this approach is constrained by the difficulty of obtaining abundant numbers of these precursor cells. This difficulty might be surmounted by establishing immortalized erythroid progenitor cell lines that are efficient producers of transfusable and functional RBCs; such cell lines represent a potentially much more valuable resource for producing RBCs than ES or iPS cell lines.

Establishment of immortalized mouse erythroid progenitor cell lines able to produce mature RBCs

In an earlier study, we developed a reliable method for establishing immortalized erythroid progenitor cell lines by the induction of hematopoietic differentiation in mouse ES cells; using this method, we established several erythroid cell lines [2]. The cell lines all exhibited the expected characteristics of erythroid cells and were designated Mouse ES cell Derived-Erythroid Progenitor (MEDEP) cell lines. The characteristics of MEDEP cell lines did not change even after long-term culture for more than 2 years [3]. Although the precise features of each line varied, all MEDEP cell lines could differentiate in vitro into more mature erythroid cells, including enucleated RBCs. To examine whether these cell lines functioned in vivo, we transplanted MEDEP cells into mice suffering from acute anemia. We found that the cells showed transient proliferation and also differentiation into functional RBCs. The treated mice showed a significant amelioration of the acute anemia. Importantly, the MEDEP cells did not form tumors following transplantation into the mice. These two reports provide the first demonstration of the feasibility of establishing immortalized erythroid progenitor cell lines able to produce mature RBCs.

At present, the mechanism by which differentiated cell lines are established from ES cells has not been elucidated. Nevertheless, our data clearly indicate that useful erythroid cell lines can be reproducibly obtained from mouse ES cells. Given that the strategies for inducing differentiation in mouse ES cells often differ from those applied to human ES cells, there is a possibility that our method for producing MEDEP cell lines might not be directly applicable to human ES cells and that some modifications will be necessary.

Can immortalized human erythroid progenitor cell lines be established from ES cells using the mouse protocol?

We applied a slightly modified version of the method used to generate MEDEP cell lines to human ES cells to determine whether immortalized human erythroid progenitor cell lines could be established (Fig. 1). Two human ES cell lines developed in Japan, KhES-2 and KhES-3, were used in
We found that it was possible to induce hematopoietic cells from the two cell lines, however, the rate of production of erythroid cells was very low in both (Fig. 2). As a result, we were unable to establish immortalized erythroid progenitor cell lines from these ES cell lines.

Can immortalized human erythroid progenitor cell lines be established from iPS cells?

Our next step was to apply the same method to human iPS cells. The human iPS cell lines used in the experiment were generated in a previous study [5]. Again, we found that the rate of production of erythroid cells was too low to allow establishment of immortalized erythroid progenitor cell lines (data not shown).

It has been reported that enforced expression of the transcription factor Tal1 in ES cells of the common marmoset (Callithrix jacchus) caused a substantial increase in the rate of hematopoietic cell differentiation [6]. To determine whether this transcription factor might also have an effect in human cells, we developed human iPS cell lines expressing Tal1 (Tal1-iPS cells). We found that the rate of hematopoietic cell production was significantly higher in the Tal1-iPS cells than their parental iPS cells. More notably, erythroid cells (Glycophorin A-positive cells) were also induced in larger numbers from the Tal1-iPS cells (data not shown).

We used the hematopoietic cells induced from Tal1-iPS cells to establish immortalized erythroid progenitor cell lines using the method described in Fig. 1. Several hematopoietic cell lines expressing CD36, a marker of erythroid
cells, were obtained (a representative cell line is shown in Fig. 3). After induced expression of Gfi1B, or GATA1 or both, Glycophorin A-positive mature erythroid cells were obtained at an abundant rate (Fig. 3); however, enucleated RBCs were not produced after induced differentiation in any of the cell lines.

Can immortalized human erythroid progenitor cell lines be produced using HPV16-E6/E7?

Enforced expression of human papilloma virus (HPV) 16-E6/E7 protein in CD36-positive erythroid cells has recently been used to generate immortalized cell lines [7]. We therefore decided to use this approach with human umbilical cord blood cells and iPS cells as the source cell materials. This method allowed successful establishment of immortalized human erythroid progenitor cell lines that were able to differentiate into very mature erythroid cells. We designated these lines as Human Umbilical Cord Blood Derived-Erythroid Progenitor (HUDEP) cell lines and Human iPS cell Derived-Erythroid Progenitor (HiDEP) cell lines. Currently, we are intensively investigating the characteristics of the HUDEP and HiDEP cell lines. Our preliminary analyses have demonstrated that all of the HUDEP and HiDEP cell lines can produce functional hemoglobin and can also produce enucleated RBCs (reticulocytes) to some degree.

Conclusion

We are now confident that we can establish immortalized human erythroid progenitor cell lines that are able to differentiate into very mature cells including hemoglobin-producing RBCs and enucleated RBCs.

Acknowledgements

We thank Dr. Kiyono for providing the HPV16-E6/E7 DNA and all members in the Cell Engineering Division for help, discussion, or secretarial assistance. This work was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology in Japan.

Disclosure

No potential conflict of interests to declare.

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