Tendency of K562 chronic myeloid leukemia cells towards cell reprogramming

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Abstract

Cancer cell reprogramming is a potential tool to study cancer progression, disease pathology and drug sensitivity. Prior to performing cancer reprogramming studies, it is important to evaluate the stemness predisposition of cells that will be reprogrammed. Here, we performed a proof-of-concept study with chronic myeloid leukemia K562 cells in order to evaluate their tendency for cancer cell reprogramming. Expression of reprogramming factors, pluripotency markers and tumor-suppressor genes were analyzed at gene and protein level via real-time RT-PCR and flow cytometry. Human PBMCs were used as positive control and K562 cells were shown to express higher levels of most of the reprogramming factors, pluripotency markers. Expression of p53, which is one of the main regulator during iPSC generation were found to be lowered in K562 cells compared to PBMCs, whereas the other tumor-suppressor genes showed higher expression levels. This study suggested that similar to healthy human PBMCs, K526 cells could be used in cancer cell reprogramming studies. Generating iPSC cells from leukemia cells could help scientists to establish CML models in vitro, better understand therapy resistance and develop novel therapeutic targets.

Keywords: induced pluripotent stem cells (iPSCs); chronic myeloid leukemia; K562; disease modeling; cell reprogramming
Introduction

Since their discovery, induced pluripotent stem cells (iPSCs) have been extensively used to model diseases and test drugs in vitro[1-6]. Hematological disorders including chronic myeloid leukemia have been modeled by various research groups[4, 7, 8]. iPSCs capture the genetic alterations present in leukemia cells and their differentiation ability helps scientists to understand disease progression and therapy resistance.

In one of the first studies, a chronic myeloid leukemia cell (CML) line KBM7 was reprogrammed towards pluripotency via retroviral vectors carrying OKSM (Oct3/4, Klf-4, Sox-2, c-Myc) factors[9]. Unlike the untreated cells, reprogrammed group showed resistance to chemotherapeutic agent imatinib, which is an inhibitor of BCL-ABL oncogene. It was hypothesized that the therapeutic agent imatinib targets cells in a specific epigenetic differentiated cell state, which can contribute to its inability to fully eradicate disease in chronic myeloid leukemia patients [10]. Later, Bedel et al. reported that when CD34BCR-ABL+ cells from CML patient were reprogrammed, CML-iPSCs lost the BCR-ABL dependency and became resistant to tyrosine kinase inhibitor therapy[11]. The authors suggested that CML-iPSC can be used to study mechanisms by which leukemic stem cells survive to therapy and is a promising tool for testing and screening new therapeutic target reducing leukemic stem cell survival[11]. In another CML study, again with the use of retroviral vectors, iPSC cells were generated from primary CML patient cells. Although CML-iPSs were resistant to the chemotherapeutic agent imatinib, CML-iPSC derived hematopoietic cells recovered sensitivity to the drug [12]. In another study, whole genome sequencing of CML derived iPSCs revealed genocopying of highly mutated primary leukemic cells which were used to understand the selective growth under tyrosine kinase inhibitor therapies [13]. In 2015, iPSCs were used to identify the leukemia stem cells for primitive CML by Suknuntha et al. Due to the rarity of leukemia stem cells in within the primitive hematopoietic cell compartment, it is difficult to study their contribution[14]. By the generation of CML-iPSCs, the authors discovered olfactomedin 4 as a novel factor that contributes to survival and growth of somatic lin(-)CD34(+) cells from bone marrow of patients with CML in chronic phase, but not primitive hematopoietic cells from normal bone marrow[14]. These contradictory results show that more work is needed to model CML. However, as in the reprogramming of healthy cells, there are various factors affecting reprogramming efficiency and for this reason, these factors should be first determined for leukemia in order to model such diseases in vitro.

As can be seen from the above studies, reprogramming cancer cells is a potential tool to study cancer progression, disease pathology and drug sensitivity. Prior to performing cancer reprogramming studies, it is important to evaluate the stemness predisposition of cells that will be reprogrammed[4]. Here, we performed a proof-of-concept study with K562 cells in order to evaluate their tendency for cancer cell reprogramming. We analyzed the endogenous expression of reprogramming and pluripotency factors that are known to be important factors for cell reprogramming. Furthermore, it is well-know that the expression of tumor suppressor genes also determine reprogramming efficiency[15]. Therefore the levels of important tumor suppressor genes are identified in K562 cells.
Materials and Methods

Cell culture

Human chronic myeloid leukemia cell line K562 and human peripheral blood mononuclear cells (PBMCs) were obtained from ATCC and Lonza respectively. Cells were cultured in RPMI1640 supplemented with 10 % fetal bovine serum (FBS), 50 U/ml penicillin, 50 μg/ml streptomycin, 1% L-glutamine at 37°C in 5 % CO₂.

RNA extraction and quantitative real time PCR (qRT-PCR)

Cells (1 x 10⁶ cells) were collected and RNA was extracted with the Machery Nagel RNA isolation kit. cDNA synthesis from 1 μg of RNA sample was performed with iScript cDNA synthesis kit (Bio-Rad) according to manufacturer’s instructions. 2 μl of each cDNA sample were used to perform real-time RT-qPCR reactions with iO SYBR Green Supermix (Bio-Rad, UK). Samples were run on CFX-96 Connect Real Time System (Bio-Rad) with the following protocol: 95°C for 3 min, 1 cycle; 95°C for 10 sec, 60°C for 30 sec, – repeated for 40 cycles. GAPDH was used as a reference gene and gene expression levels for OCT3/4, SOX2, KLF4, CMYC, NANOG, REX, CRIP1TO, P53, P21, P16, PRB were normalized to PBMCs.

Flow cytometry analysis

Cells (1 x 10⁶ cells) were collected by centrifugation, washed with ice cold methanol for fixation and then with permeabilized with 0.1 %Triton-X100 containing 2% BSA-PBS. Following washing with PBS, cells were stained with rabbit anti-Oct3/4, rabbit anti-Nanog or mouse anti-p53 antibodies. Anti-rabbit-AF543 or anti-mouse-AF488 were used as secondary antibodies. Cells were analyzed in BD Accuri Plus Flow Cytometer (BD). For 10000 events, percentages of positive populations were determined by using BD Accuri Plus software (BD).

Statistical Analysis

Triplicates containing required amount of cells were used during analyses. Delta Ct values were used for the statistical analysis of RT-PCR results. Statistical analysis was performed by analysis of variance and Tukey's pairwise comparison using SPSS software, version 16.0.

Results

In order to test the tendency of K562 cells towards cell reprogramming, we used human peripheral blood mononuclear cells as positive control. Since there are studies that have showed successful reprogramming with PBMCs[16], they are used as positive control in this study.

As shown in Figure 1, the reprogramming factors (Oct3/4, Klf2, Sox2, cMyc) were all upregulated in K562 cells, compared to PBMCs (Figure 1A). Significant differences were
observed for the Klf2, Sox2, cMyc genes. When the cells were analyzed for the expression of pluripotency markers including Nanog, Rex and Cripto via real-time RT-PCR, we observed higher expression levels compared to PBMCs (Figure 1B). However we obtained a lower profile when compared to the that of programming factors.

In addition to the programming and pluripotency factors, the expression of tumor suppressor genes determine the efficiency of reprogramming[15]. For this reason, we analyzed the expression of P53, P21, P16 and PRB genes and found that P53 is downregulated in K562 cells compared to PBMCs, whereas the others showed higher expression levels (Figure 2).

In order to confirm the gene expression data, we performed flow cytometric analysis of programming factor Oct3/4, pluripotency marker Nanog and tumor suppressor p53 in PBMCs and K562 cells. Flow cytometry analyses confirmed the real-time RT-PCR data. The percentage of positive cells for Oct3/4 and Nanog were increased to 11.7% and 9.5%, respectively (Figure 3). When anti-P53 antibodies were used to stain the cells in flow cytometry, in contrast to real-time RT-PCR data, there was no significant change in the P53 positive cell populations (Figure 4). This may suggest that even though there was a difference at the mRNA level, protein levels do not vary, possibly due to posttranscriptional regulation.

Discussion

It has been previously reported that the expression of reprogramming and pluripotency factors in the starting cells are limiting factors in cell reprogramming[4, 17]. As shown here, higher levels reprogramming and pluripotent factors both at gene and protein levels, increase their tendency towards cellular reprogramming. On the other hand, expression of tumor suppressor genes needs to be controlled during iPSC generation[15, 18, 19]. In this study, we observed down-regulation of P53 mRNA and similar levels of its protein in K562 cells compared to PBMCs. However the overall expression of other tumor-suppressor genes can be still limiting factors. Therefore the expression of these genes should be carefully monitored during reprogramming. Silencing strategies could be needed to achieve efficient reprogramming. Until now, cancer reprogramming studies for CML has not focused on the expression of above factors[9, 11, 13, 20, 21] and there is no study that have reported the link between these factors and the ease of reprogramming. Therefore this is an important preliminary study that reinforces the importance of these factors.

Screening the levels of reprogramming and pluripotency factors have been one of the ways to assess the efficiency of iPSC generation[4]. On the other hand, expression of these markers has been also linked with multidrug resistance of leukemia cells through modulating the ATP-binding-cassette transporters (ABC-transporters)[9, 21]. For example, the expressions of Oct4, Sox2, and Nanog, all of which are studies in the present study, have been shown to be upregulated in K562 cells when they retain multidrug resistance to doxorubicin[20, 22]. Therefore this also suggests that monitoring their expression status is a key step in order to model the disease and study drug resistance.
In addition to the above factors, there are other factors that limit the efficiency of iPSC generation from cancer cells. These could be listed as the proliferation rate of cancer cells, epigenetic background, long term culturing conditions during reprogramming, heterogeneity of tumor cells and the presence of cancer stem cells[4]. For example, highly proliferating cells will be difficult to reprogram since dividing cancer cells and reprogrammed cancer cells will compete in the culture condition which would not allow for ground state pluripotency in the reprogrammed cells[23]. Therefore future studies should monitor the above parameters in detail during cell reprogramming of CML.

Considering the PBMC usage as the cell source in reprogramming protocols, this proof-of-concept study showed that, K526 chronic myeloid leukemia cells could be also used in cancer cell reprogramming studies. Generating iPS cells from these cell lines could help scientists to establish CML models in vitro. This can allow us to study disease progression, drug responses and disease pathology.

Conclusion

This study suggested that similar to healthy human PBMCs, K526 cells could be used in cancer cell reprogramming studies. Generating iPS cells from leukemia cells could help scientists to establish CML models in vitro, better understand disease progression and develop novel therapeutic targets.

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References

**Figure Legends**

**Figure 1. Gene expression of reprogramming factors and pluripotency markers.** RNA was isolated from PBMC and K562 cells and qRT-PCR was performed. Relative gene expression was plotted for A) reprogramming factors and B) pluripotency markers. GAPDH was used reference gene, data was normalized to PBMC. *p<0.05 compared to PBMC.

**Figure 2. Gene expression of tumor suppressor genes.** RNA was isolated from PBMC and K562 cells and qRT-PCR was performed. Relative gene expression was plotted for tumor suppressor genes. GAPDH was used reference gene, data was normalized to PBMC. *p<0.05 compared to PBMC. Small graph shows the gene expression profile of p53 and p16 in order to better represent the differences.

**Figure 3. Protein expression of reprogramming factor Oct3/4 and pluripotency marker Nanog.** Cells were collected by centrifugation, followed by staining for Oct3/4 and Nanog. Cells were analyzed in BD Accuri Plus Flow Cytometer (BD). *p<0.05 compared to PBMC.

**Figure 4. Protein expression of tumor suppressor gene p53.** Cells were collected by centrifugation, followed by staining for p53. Cells were analyzed in BD Accuri Plus Flow Cytometer (BD).
Figure 1

**A**

Normalized Gene Expression

- Oct3/4
- Klf4
- cMyc
- Sox2

**B**

Normalized Gene Expression

- Nanog
- Rex
- Cripto

PBMNC
K562
Figure 2
Figure 4