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Original Article

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Gene Expression Analysis of SOX2, NANOG, KLF4, OCT4, and REX1 Genes in Cord Blood Mononuclear Cells Treated with External Stresses

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Abstract

Background: *Induced pluripotent stem cells (iPSCS) can be obtained from autologous cells for therapeutic purposes. So, far, many studies have been done to produce induced pluripotent cells by transferring specific pluripotency genes using different methods. In this study, pluripotency gene expression induced by external stresses was assessed in cord blood mononuclear cells.*
Methods: *In this experimental study, mononuclear cells were isolated from umbilical cord blood. Isolated cells were divided into three groups. The first group had been exposed to HCL (pH 5.7) for 25 minutes and then transferred to the medium with normal pH. The second group was triturated with hamilton syringe for 15 min (external pressure), and the last group was considered the control group and did not receive treatment. Then, total RNA was extracted on Day 7. Gene expression of OCT4, SOX2, NANOG, REX1, and KLF4 was evaluated using qRT-PCR.*
Results: *Gene expression of OCT4, NANOG, REX1, and KLF4 was increased after exposure to acidic pH and external pressures in comparison with control cells ($P < 0.05$). SOX2 gene expression was decreased in cells exposed to acidic pH but increased by external pressure.*
Conclusions: *Exposure of umbilical cord blood mononuclear cells to acidic pH and external pressure lead to re-activation of pluripotency genes in mature cells. These findings indicate that mature cells may be reprogrammed with manipulation of environmental conditions.*

Keywords: Reprogramming, External pressure, Acidic pH, Mononuclear cells, Cord blood.
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Introduction

The innovation of induced pluripotent stem cells (iPSCs) by Yamanaka and Kazutoshi Takahashi in 2006 revolutionized the field of stem cell biology.[1] For the first time, pluripotent stem cells could be acquired not only from the inner cell mass of the blastocyst-stage embryo but also from reprogramming somatic adult tissue by enforced expression of Oct4, Klf4, Sox2, and c-Myc (OKSM).[2,3] While the potential applications for human iPSCs are largely similar to human embryonic stem cells, the ethical issues and immune rejections in transplantations lead us to use non-autologous embryonic stem cells.[4] iPSCs have the capacity to differentiate into different cell lineages and even human organisms.[5] Therefore, iPSCs can ultimately be used for both disease modeling in vitro and the production of cellular products in regenerative medicine.[6]

There are many methods for production of iPSCs, categorized as either main techniques such as viral-integrating, or non-integrating techniques which are presented for the production of iPS cells.[7] The non-integrating reprogramming techniques include both DNA (such as adenoviruses and episomal plasmid vectors) and DNA-free (such as protein or peptide-based delivery and mRNA or microRNA) techniques.[8] Though these strategies have some advantages the primary concern is their lack of efficiency. Retroviral integration causes instability of genomes and increases the risk of tumorgenicity; consequently, this method is not used in clinical settings.[9] Among physical stresses, hyperosmotic shock induced by high glucose could lead to expression of pluripotency genes such as KLF-4, SOX-2, Nanog and OCT-4.[10] Such physical stress could be applied in the form of external pressure as mentioned by Obokata et al.[11] In this research, to study the influence of external stresses on pluripotency- genes expression, mononuclear cells from cord blood were treated with acidic pH and physical pressure.

Materials and Methods

This study was approved by ethical committee of Tarbiat Modares University (52D/3695). Umbilical cord blood (UCB) was obtained from Iranian Blood Transfusion Organization. Mononuclear cells (MNCs) were isolated from UCB by Ficoll-Paque PLUS (GE Healthcare) density gradient centrifugation. Then, cells were cultured in Dullbeco Medium Eagrl's Modified (DMEM)/F12 supplemented with L-glutamine (GIBCO), 12.5% inactivated Fetal Bovin Serum (FBS), 1% non-essential amino acids (Sigma-Aldrich), 100 unit µg/ml penicillin, 100 µg/ml streptomycin (GIBCO) and bFGF (GIBCO) 20ng/ml.

MNCs were divided into 3 groups. The first group was the control without any manipulation. The second group was exposed to acidic pH (pH 5.7) for 25 min at 37°C. Then, medium was exchanged with normal pH. The third group was triturated with Hamilton syringe (gage 32) for 15 min by gentle flushing. Flasks were incubated at 37°C for 7 days. The cells were counted and stained by trypan blue.

Table 1. Sequences of primers were used for quantitative RT- PCR

Genes	Primer sequence
Sox2	F 5′ggactgagagaaagaagagga -3′ R 5′-gaaaatcaggcgaagaataat -3′
NANOG	F 5′-aatggtgtgacgcagggatg -3′ R 5′-tgcaccaggtctgagtgttc -3′
β-actin	F 5′-gtcctctcccaagtccacac - 3′ R 5′-gggagaccaaaagccttcat - 3′
REX1	F 5′-cgggacgaggagtgttattac -3′ R 5′-cgtgttgctttgcgacttg -3′
Klf4	F 5′-taccaagagctcatgccacc -3′ R: 5′- gcgaatttccatccacagcc-3′
OCT-4/POU5f1	F: 5′-cgccgtatgagttctgtg -3′ R: 5′-ggtgatcctcttctgcttc -3′

Total RNA was isolated with the Trizol (Invitrogen). Reverse transcription was performed using cDNA synthesis kit (Fermentase). The cDNA was subjected to qRT-PCR using Power SYBR Green Master Mix (Applied Biosystems). β-actin in each sample was quantified as an endogenous control. The relative expression levels of OCT4, NANOG, REX1, KLF4 and SOX2 were calculated using the $2^{-\Delta\Delta Ct}$ method. The sequence of primers has been described in Table1.

All tests were carried out in triplicate. Data means were compared using one-way ANOVA. Statistical analyses were performed by SPSS version 16.0 analytical software. Statistical significance was defined at $P < 0.05$.

Results

Adherent cell clusters were observed in flasks containing treated cells with acid and external pressure (Hamilton syringe) after 5 days. (Figure 1 and Figure 2)

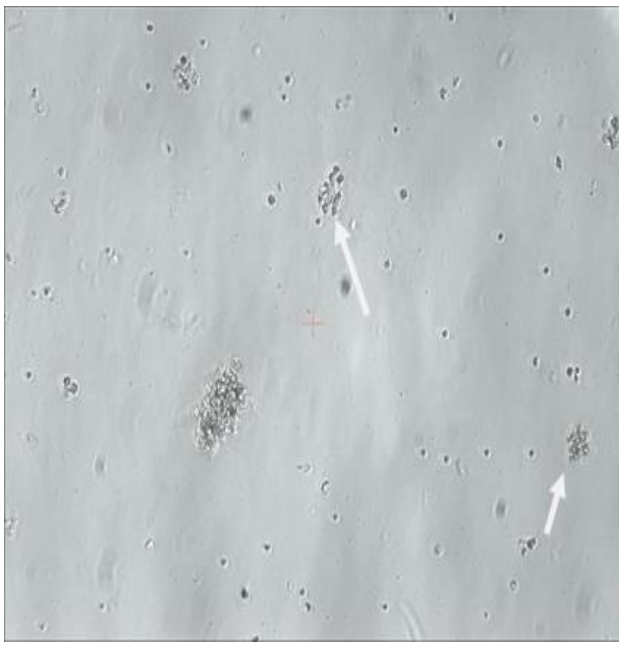

Figure 1. Forming of cell clusters under the acidic condition and pressure after 5 days. Magnification: 400X

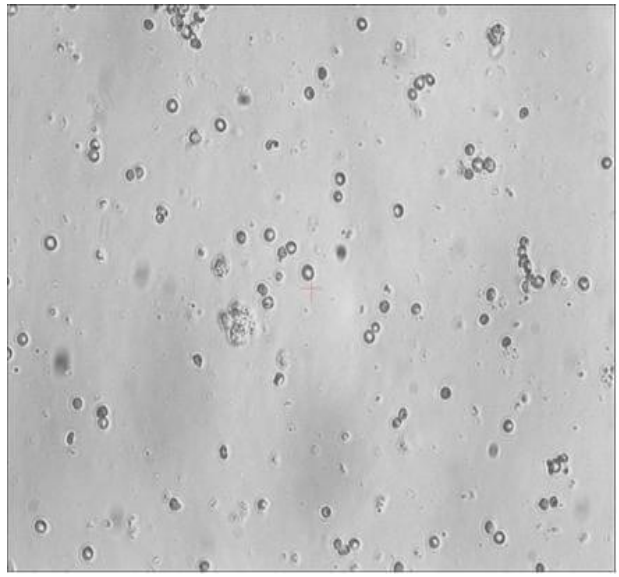

Figure 2. Formation of cell clusters under pressure after 5 days. Magnification: 400×

Quantitative analysis of OCT4 gene expression showed the expression of this gene in cells exposed to acidic pH was 6.1±1.59 folds (P = 0.001), and in cells exposed to external pressure it was 3.01±0.76 folds (P = 0.004) in comparison with control group. (Figure 3)

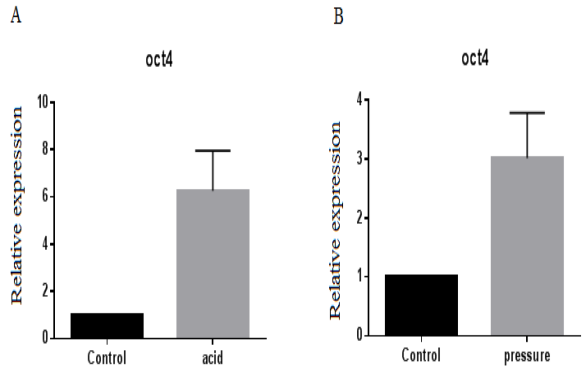

Figure 3. qRT-PCR gene expression analysis of OCT4 (A) exposed to acidic condition; (B) external pressure.

Quantitative analysis of KLF4 gene expression showed the expression of this gene in cells exposed to acidic conditions was 3.9±1.14 folds (P = 0.01) and in cells exposed to external pressure was 6.98±1.07 folds (P < 0.001) in comparison with control group. (Figure 4)

Quantitative analysis of Nanog gene expression showed the expression of this gene in cells exposed to acidic conditions was 2.5±0.78 folds (P = 0.03) and in cells exposed to external pressure was 7.5±1.56 folds (P = 0.004) in comparison with control group. (Figure 5)

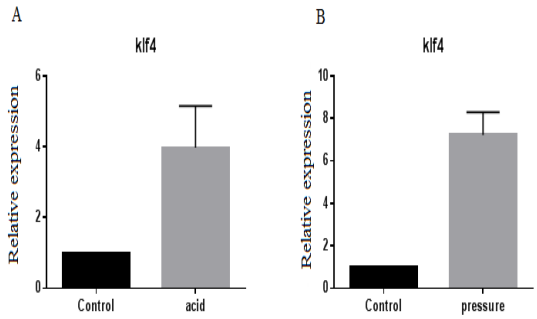

Figure 4. qRT-PCR gene expression analysis of KLF4 (A) exposed to acidic condition; (B) external pressure.

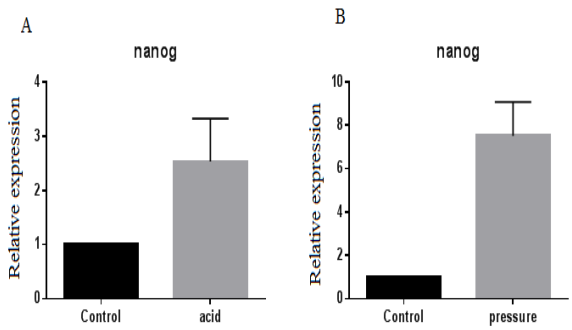

Figure 5. qRT-PCR gene expression analysis of NANOG (A) exposed to acidic condition; (B) external pressure.

Quantitative analysis of REX1 gene showed the expression of this gene in cells exposed to acidic conditions was 5.06±2.13 folds (P = 0.01) and in cells exposed to external pressure was 2.40±0.79 folds (P = 0.01) in comparison with control group. (Figure 6)

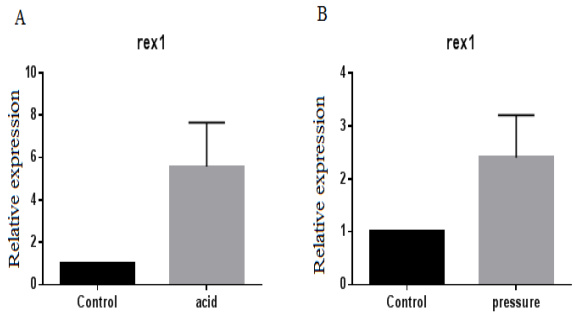

Figure 6. qRT-PCR gene expression analysis of REX1 (A) exposed to acidic condition; (B) external pressure.

Quantitative analysis of SOX2 gene showed the expression of this gene in cells exposed to acidic conditions was 0.1928±0.093 fold (P < 0.001) and in cells exposed to external pressure was 4.738±1.27 fold (P = 0.002) in comparison with control group. (Figure 7)

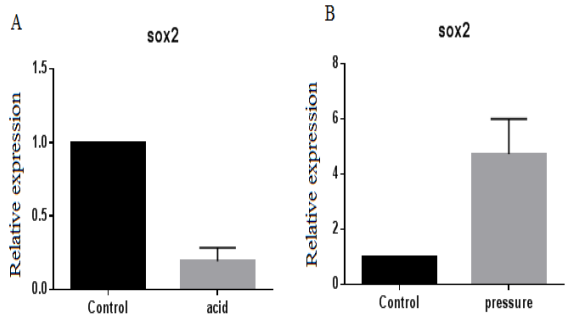

Figure 7. qRT-PCR gene expression analysis of SOX2 (A) exposed to acidic condition; (B) external pressure.

Discussion

Despite the advantages for ESCs, there are several limitations, such as ethical issues and immune rejections in transplantations which are restricting the use of these cells in curing diseases.[8] On this subject, in 2006 a major revolution was achieved in iPSCs.[9] The potential use of iPSCs in regenerative medicine and treatment of many disorders such as genetic diseases receives the most attention.[12,13] MNCs include monocytes, lymphocytes and a small number of progenitor cells. Myeloid or erythroid progenitor cells are the desirable cell type for reprogramming due to their completeness in the genome and robust plasticity potential for cell fate conversion.[14] A current study showed cord blood MNCs could convert to iPSCs with high efficiency.[15] This study investigated cell reprogramming genes through the expression of pluripotency genes happened in mature cells under environmental conditions. In 2014, Haruko Obakata[11] surveyed influence of difference levels of lethal stress on mouse CD45+ cells in mice. That study has showed that somatic cells latently possess a surprising plasticity. This dynamic plasticity, the ability to become pluripotent cells, emerges when cells are transiently exposed to strong stimuli that they would not normally experience in their living environments. In that study, the cells were tested under different treatments. Among these treatments, the most responsive were acidic pH (5.4–5.7) and after that, it was particularly physical damage by rigorous trituration and membrane perforation by Strepto-Lysin O.[11] Acidic conditions were chosen similar to Obakata et al., but trituration was a little different, physical damage was done by Hamilton syringe (32gage). In Obakata's study, using quantitative polymerase chain reaction (qPCR) analysis, most of the population expressed pluripotency genes but NANOG and REX1 did not.[11] In our study, gene expression of NANOG, OCT4, REX1 and KLF4 were increased following treatment with both external stresses. Expression of SOX2 gene in treated cells with acidic pH was decreased but in treated cells with pressure, SOX2 overexpression was observed. Down regulation of SOX2 gene expression may be because of apoptosis process induced in treated cells.[16]

Our study showed that we may use a combination of different conditions to induce reprogramming in mature cells. It is possible that the mechanism of activation of pluripotency genes in cells under acidic condition is similar to the cells are under hypoxic condition which medicated by HIF-2.[17,18] Further experiments are needed to determine precise mechanism of pluripotency genes re-activation in somatic cells induced with external stimulus.

We showed the ability of cord blood derived MNCs to express high levels of pluripotency gene expression which are transiently exposed to external chemical and physical stress. Totally, our study showed the novel method to produce transient pluripotency in somatic cells by changing environmental conditions instead of genetic manipulation. Further investigations are needed to expand our results.

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Conflict of Interest

The authors declare that They have no conflict of interest.

References

1. Yamanaka S. Induced pluripotent stem cells: past, present, and future. Cell Stem Cell 2012;10:678–84. doi:10.1016/j.stem.2012.05.005

2. Mah N, Wang Y, Liao MC, Prigione A, Jozefczuk J, Lichtner B, et al. Molecular Insights into Reprogramming-Initiation Events Mediated by the OSKM Gene Regulatory Network. PLoS One 2011;6:1-17. doi:10.1371/journal.pone.0024351

3. Parenti A, Halbisen MA, Wang K, Latham K, Ralston A. OSKM Induce Extraembryonic Endoderm Stem Cells in Parallel to Induced Pluripotent Stem Cells. Stem cell reports 2016;6:447–55. doi:10.1016/j.stemcr.2016.02.003

4. Tavernier G, Mlody B, Demeester J, Adjaye J, De Smedt SC. Current methods for inducing pluripotency in somatic cells. Adv Mater 2013;25:2765–71. doi:10.1002/adma.201204874

5. Huang G, Ye S, Zhou X, Liu D, Ying Q-L. Molecular basis of embryonic stem cell self-renewal: from signaling pathways to pluripotency network. Cell Mol Life Sci 2015;72:1741–57. doi:10.1007/s00018-015-1833-2

6. Lorenzo IM, Fleischer A, Bachiller D. Generation of mouse and human induced pluripotent stem cells (iPSC) from primary somatic cells. Stem Cell Rev 2013;9:435–50. doi:10.1007/s12015-012-9412-5

7. Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science 2009;324:797–801. doi:10.1126/science.1172482

8. Wang J, Gu Q, Hao J, Bai D, Liu L, Zhao X, et al. Generation of induced pluripotent stem cells with high efficiency from human umbilical cord blood mononuclear cells. Genomics Proteomics Bioinformatics. 2013;11:304–11. doi:10.1016/j.gpb.2013.08.002

9. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–76. doi:10.1016/j.cell.2006.07.024

10. Madonna R, Görbe A, Ferdinandy P, De Caterina R. Glucose Metabolism, Hyperosmotic Stress, and Reprogramming of Somatic Cells. Mol Biotechnol [Internet]. 2013;55(2):169–78. Available from: doi:10.1007/s12033-013-9668-2

11. Obokata H, Wakayama T, Sasai Y, Kojima K, Vacanti MP, Niwa H, et al. Stimulus-triggered fate conversion of somatic cells into pluripotency. Nature 2014;505:641–7. doi:10.1038/nature12968

12. Singh VK, Kalsan M, Kumar N, Saini A, Chandra R. Induced pluripotent stem cells: applications in regenerative medicine, disease modeling, and drug discovery. Front cell Dev Biol 2015;3:1-18. doi:10.3389/fcell.2015.00002

13. Avior Y, Sagi I, Benvenisty N. Pluripotent stem cells in disease modelling and drug discovery. Nat Rev Mol Cell Biol 2016; 17:170-82. doi:10.1038/nrm.2015.27

14. Su RJ, Neises A, Zhang X-B. Generation of iPS cells from human peripheral blood mononuclear cells using episomal vectors. Methods Mol Biol 2016;1357:57–69. doi:10.1007/7651_2014_139

15. Liu SP, Li YX, Xu J, Gu HH, Zhang HY, Liang HY, et al. An improved method for generating integration-free human induced pluripotent stem cells. J Exp Hematol Assoc Pathophysiol 2014;22:580–7. doi:10.7534/j.issn.1009-2137.2014.03.002

16. Pietrobono S, Morandi A, Gagliardi S, Gerlini G, Borgognoni L, Chiarugi P, et al. Down-regulation of SOX2 underlies the inhibitory effects of the triphenylmethane gentian violet on melanoma cell self-renewal and survival. J Invest Dermatol 2016;136:2059-69. doi:10.1016/j.jid.2016.06.610

17. Bertout JA, Majmundar AJ, Gordan JD, Lam JC, Ditsworth D, Keith B, et al. HIF2α inhibition promotes p53 pathway activity, tumor cell death, and radiation responses. Proc Natl Acad Sci 2009;106:14391–6. doi:10.1073/pnas.0907357106

18. Covello KL, Kehler J, Yu H, Gordan JD, Arsham AM, Hu C-J, et al. HIF-2α regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes Dev 2006;20:557–70. doi:10.1101/gad.1399906.