BMB Reports - Manuscript Submission

Manuscript Draft

Manuscript Number: BMB-16-055

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Article Type: Perspective (Invited Only)

Keywords: somatic cell nuclear transfer; pluripotent stem cells; therapeutic cloning; HLA-matched; stem cell bank

Corresponding Author: Dong Ryul Lee

Authors: Jeoung Eun Lee[1], Young Gie Chung[1], Jin Hee Eum[1], Dong Ryul Lee[1,*]

Institution: [1]CHA Stem Cell Institute, CHA University, Seongnam 13488, Korea, [2]Research Institute for Stem Cell Research, CHA Health Systems, Los Angeles, CA 90036, USA,
[3]Department of Biomedical Science, CHA University, Seongnam 13488, Korea,

Perspective

Highly Efficient SCNT Technology Supports Establishment of Human Personalized and Public pluripotent Stem Cell Bank

Jeoung Eun Lee[1] Young Gie Chung[2], Jin Hee Eum[1] and Dong Ryul Lee[1,2,3,*]

[1] CHA Stem Cell Institute, CHA University, Seongnam 13488, Korea

[2] Research Institute for Stem Cell Research, CHA Health Systems, Los Angeles, CA 90036, USA

[3] Department of Biomedical Science, CHA University, Seongnam 13488,, Korea

[*] Corresponding author. E-mail:drleedr@cha.ac.kr

Running Title: SCNT contributes a stem cell bank

Keywords: somatic cell nuclear transfer; pluripotent stem cells, therapeutic cloning, HLA-matched, stem cell bank, ESC derivation

Abbreviations: SCNT, somatic cell nuclear transfer; H3K9me3, histone H3 lysine 9 trimethylation; iPS, induced pluripotent stem cell; HLA, human leukocyte antigen;

Perspective to: Young Gie Chung et al (2015), Histone Demethylase Expression Enhances Human Somatic Cell Nuclear Transfer Efficiency and Promotes Derivation of Pluripotent Stem Cells, Cell Stem Cell, 17(6):758-66. doi: 10.1016/j.stem.2015.10.001.

Abstract: Although three different research groups have reported the successful derivation of human somatic cell nuclear transfer-derived embryonic stem cells (SCNT-ESCs) using fetal, neonatal and adult fibroblasts, their extremely low efficiency of human blastocyst formation would be a main limitation for the potential application. Recently, our group has found that H3K9me3 in the human somatic cell genome is an SCNT reprogramming barrier and overexpression of KDM4A, a related H3K9me3 demethylase, significantly improves the blastocyst formation of SCNT embryos by facilitating transcriptional reprogramming. Since several approaches for the optimization of SCNT conditions such as the use of protein phosphatase inhibitors, oocyte activation method and epigenetic regulation have been applied in order to improve the efficiency, it may be a powerful method for establishment of the stem cell bank with pluripotent stem cells for public supply as well as personalized stem cells.

Embryonic stem cells (ESCs) by somatic cell nuclear transfer (SCNT) can provide a research model for studying the mechanism of diseases and a patient specific-cell therapy strategy for the treatment of incurable disease. Recently, several independent research groups have reported the establishment of human SCNT-ESC (Tachibana *et al*. (2013) Cell 153(6):1228-38. doi: 10.1016/j.cell.2013.05.006; Chung *et al*. (2014) Cell Stem Cell 14(6):777-80. doi: 10.1016/j.stem.2014.03.015; Yamada *et al*. (2014) Nature 510(7506):533-6. doi: 10.1038/nature13287). However, reprogramming of human somatic cells through SCNT is still not efficient, because the extremely low rate of SCNT embryos was developed to the blastocyst and established to the ESC. In 2014, through comparative transcriptomic and epigenomic analyses of mouse *in vitro* fertilization (IVF) and SCNT embryos, Zhang and his colleagues have identified one of intrinsic epigenetic barriers, H3K9me3, that prevent somatic cell nuclear reprogramming and SCNT embryo development (Matoba *et al*. (2014) Cell 159(4):884-95. doi: 10.1016/j.cell.2014.09.055). In order to overcome such reprograming barriers, they applied mRNA injection of mouse Kdm4d, an H3K9me3 demethylase, during SCNT procedure, resulted in highly increased efficiency of cloned embryonic development. So, Zhang's and our groups have performed comparative transcriptomic and epigenomic analyses of human *in vitro* fertilization (IVF) and SCNT embryos, and found that H3K9me3 is also a barrier for human SCNT reprogramming. For overcoming this barrier, we performed SCNT procedure using donated human oocytes and skin fibroblasts donated from adult patients, and then mRNA of human KDM4A was injected into reconstituted eggs for overexpression of H3K9me3 demethylase. mRNA-injected reconstituted eggs have shown recovered transcriptional pattern at 8-cell stage of human embryonic genome activation (EGA) and significantly higher developmental rate up to blastocyst when compared with mRNA-non-injected those. Using this improved SCNT method, we successfully established multiple functionally normal SCNT-ESC lines, while none of the control (mRNA-non-injected) embryos established to ESC line. More importantly, establishment of SCNT-ESC in the study was not limited to a particular oocyte donor. In fact, it has been well known that only oocytes with the highest quality from certain females can support the development of SCNT embryos to the blastocyst stage. Therefore, when injection of KDM4A mRNAs is introduced for the efficient SCNT procedure, it may be suggested that practical problems such as oocyte donor's variation and shortage of useable donor pool could be overcome.

To accomplish their therapeutic goals as a possible cell sources, PSC-derived transplanted cells must not be rejected by the patient's immune system. For this, the use of autologous PSC obtained from SCNT or iPS technology is theologically ideal solution for avoiding or minimizing immune response. However, establishing autologous PSC when such cells are needed for each patient would be less economic, time consuming and technically difficult procedures. Recently, another option is suggested that the establishment of PSC bank with homozygous HLA genotypes would be very helpful. It was suggested that one PSC derived from HLA homozygous donor can provide cells for various types of patients with the HLA haplotype. Based on the theory, research teams from the UK and Japan reported that 150 and 140 HLA-homozygous-iPSCs could match 93% of the UK and 90% of the Japanese population. In addition, derivation efficiency of SCNT-ESC are remarkably increased during last decades of researches, we now suggest that this technology could be applicable to establishing PSC bank with homozygous HLA genotypes, even different mitochondrial DNA originated from donate oocytes in SCNT-ESCs could be the sources of weak and amenable allogeneic immune reaction. And, above all things, several differences of epigenetic and functional properties between iPSC and SCNT-ESC have

been observed in both mouse and human systems.

In summary, in the our recent studies, we have established an improved SCNT method and generated multiple SCNT-ESCs derived from normal with various age and patients with several types of diseases for therapeutic cell replacement. In addition, this technology will provide an opportunity to treat mitochondrial DNA-related diseases because SCNT-ESCs have different mitochondrial DNA originated from donate oocytes. Also, based on recently improved efficiency, we may suggest that this technology could be used for establishment of HLA-matched homozygous SCNT-ESC, the sources for public PSC bank based on the known donor and recipient HLA types.

ACKNOWLEDGEMENTS

This work was supported by grants from the Bio & Medical Technology Development Program (2012M3A9C6049723 and 2015M3A9C6028961) of NRF and MSIP of Republic of Korea.