Effects of Chemicals on the Cell Cycle Synchronization of Porcine Induced Pluripotent Stem Cells

Hyeon Yang, Dae-Jin Kwon[1], In-Sul Hwang, Tae-Uk Kwak, Mi-Ryung Park, Sun-A Ock, Keon Bong Oh, Jae-Seok Woo, Gi-Sun Im, Seongsoo Hwang

Animal Biotechnology Division, National Institute of Animal Science, Jeonbuk 55365, Republic of Korea
[1]International Agricultural Development and Cooperation Center, Chonbuk National University, Jeonbuk 54896, Republic of Korea

I. Introduction

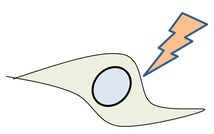
- Pluripotent stem cells may be alternative cell sources for creating transgenic animals, because they can be accessed more easily the homologous recombination, as well as cloned more efficiently than any other cell types as reported in mouse.
- Unlike mouse results, cloning efficiency of nuclear transfer from porcine induced pluripotent stem cells (piPSCs) is very low.
- Therefore the piPSCs need to be synchronized at G1 phase before using them as a nuclear donor reconstructed with MII recipient oocyte.

II. Objective

- The present study was performed to investigate the effect of cell cycle inhibitors on the cell cycle synchronization of piPSCs generated using combination of six human transcriptional factors.

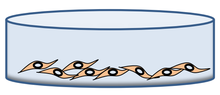
III. Materials & Methods

Treatments of iPSCs

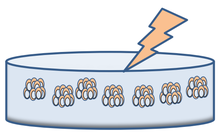


Lentiviral transduction : viPS Vector Kit
Human factors
- OCT4, NANOG, SOX2, C-MYC, KLF4, and LIN28

Seeding onto Matrigel
- Mitiomycin-treated mouse embryonic fibroblasts (MEFs)
- Transduced cell concentrations: 1 x 10^5 cells/35mm dish
- 10% FBS/KSR, 20 ng bFGF and 20 ng LIF in DMEM/F12



Colony pick-up & Passage
- 10% FBS/KSR, 20 ng bFGF and 20 ng LIF in DMEM/F12



For cell cycle synchronization treatments for 12 h, respectively.
- staurosporine (STA, 20 nM)
- daidzein (DAI, 100 uM)
- roscovitine (ROSC, 10 uM)
- olomoucine (OLO, 200 uM)

Characterization (iPSC)	Gene Expression (Viability & Apoptosis)	Gene Expression (Stem cell marker)

IV. Results

Table 1. Effects of chemical treatments on the cell cycle distribution of porcine induced pluripotent stem cells

	G0/G1	S	G2/M
Control	37.5 ± 0.2^{b}	34.0 ± 0.6^{a}	28.5 ± 0.4^{b}
Staurosporine	35.9 ± 0.9^{b}	35.2 ± 0.6^{a}	28.8 ± 1.0^{ab}
Daidzein	41.9 ± 0.9^{a}	26.1 ± 0.9^{b}	32.0 ± 2.3^{ab}
Roscovitine	32.6 ± 1.9^{bc}	33.7 ± 1.5^{a}	33.7 ± 1.8^{ab}
Olomoucine	30.3 ± 1.5^{c}	33.1 ± 2.3^{a}	36.6 ± 4.4^{a}

Mean values ± SD of the cell percentage in each cell cycle compartment measured in at least three independent experiments are reported.
^{a,b,c} Values with different superscripts are significantly different (P<0.05).

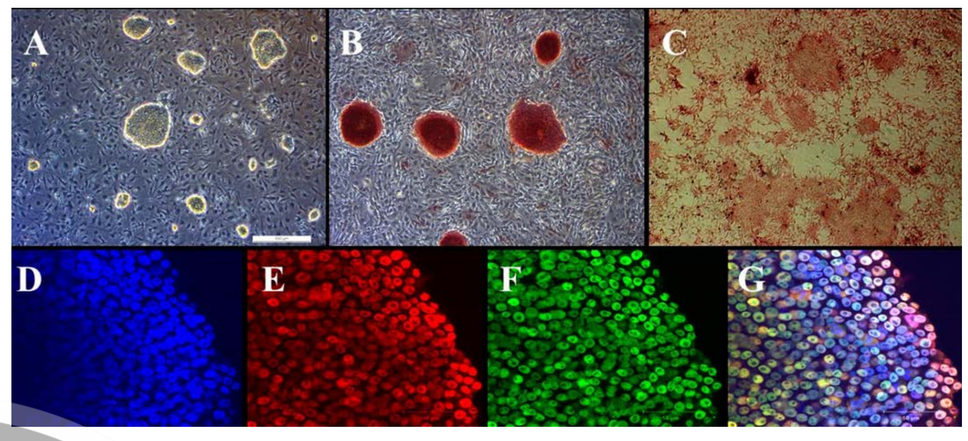


Figure 1. Morphologies and pluripotent gene expression of porcine induced pluripotent stem cells
Colonies show similar morphology to mouse ESCs (A) and they are positive for alkaline phosphatase (AP) in both feeder (B) and feeder-free (C) culture conditions. Stem cell markers, OCT4 (E) and NANOG (F) were highly expression in piPSC colony. D; DNA, G; merged image. Scale bars indicate 500 um in A-C and 50 um in D-G, respectively.

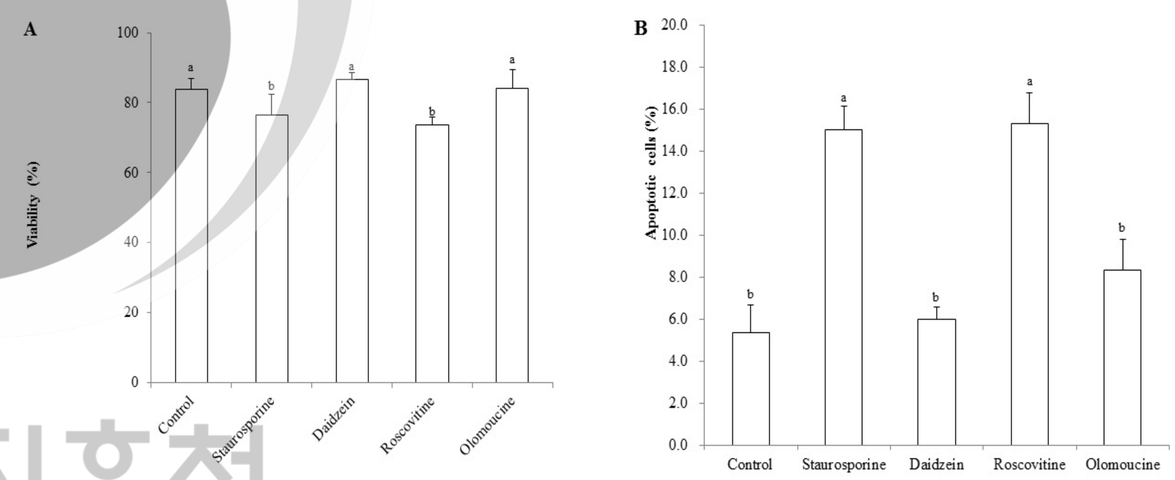


Figure 2. Effects of chemical treatments on cell viability (A) and apoptosis (B) of porcine induced pluripotent stem cells
piPSCs were treated with staurosporine (STA, 20 nM), daidzein (DAI, 100 uM), roscovitine (ROSC, 10 uM) or olomoucine (OLO, 200 uM) for 12 h, respectively.
$^{a, b}$ P<0.05.

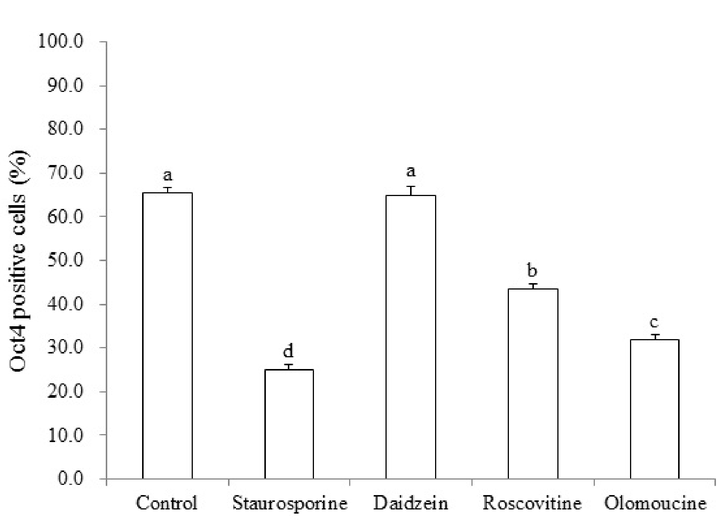


Figure 3. Effects of chemical treatments on Oct4 expression of porcine induced pluripotent stem cells
piPSCs were treated with staurosporine (STA, 20 nM), daidzein (DAI, 100 uM), roscovitine (ROSC, 10 uM) or olomoucine (OLO, 200 uM) for 12 h, respectively.
$^{a, b, c, d}$ P<0.05.

V. Conclusion

- Our results suggest that DAI could be used for synchronizing piPSCs at G1 stage and has any deleterious effect on survival and pluripotency sustaining of piPSCs.

– This work was carried out with the support by the Agenda Program (PJ01095601), Rural Development Administration, Republic of Korea.