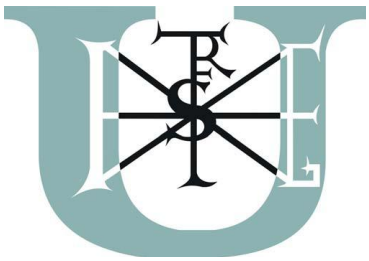

SZENT ISTVÁN UNIVERSITY

Animal Husbandry Science PhD School

GENERATION OF MOUSE INDUCED PLURIPOTENT STEM (iPS) CELLS BY *SLEEPING BEAUTY* (SB) TRANSPOSON

Thesis of the Doctoral (PhD) Dissertation

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The PhD program

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1. INTRODUCTION AND GOALS

1.1 Introduction

Embryonic stem (ES) cells have an unlimited expansion potential and are able to produce many differentiated and functional cell types. Directed differentiation of ES cells can provide a valuable source of specialized cells for regenerative cell therapy of damaged tissues. However the generation of human ES cell lines requires the sacrifice of human embryos. Also, the generation of patient-specific ES cells requires access to high quality human oocytes. Thus, the generation of human ES cells, raises serious ethical issues.

Recent advances of induced pluripotency in adult mouse and human fibroblast cells have resulted in the generation of a new type of stem cell, called induced Pluripotent Stem (iPS) cells (Takahashi and Yamanaka 2006; Wernig *et al*. 2007; Woltjen *et al*. 2009). IPS cells have been produced from adult differentiated cells in mouse and human by transgenic modification using a few key pluripotency genes. These cells have shown characteristics surprisingly similar to ES cells and like ES cells, are also able to differentiate into many somatic tissues. To date, the best demonstration of their ES-like differentiation potential has been through the generation of whole mice by tetraploid complementation (Zhao *et al*. 2009). iPS cells might be a replacement for ES cells, as they overcome the ethical and legal limitations of embryo and cloning research. The long-term goal of iPS technology is to generate patient-specific donor cells for transplantation, which can be expanded and differentiated to multiple cell types, and also be genetically modified for gene therapy purposes.

Despite the success with retro- and lentivirus based iPS cell generation, there are reports on increased prevalence of tumor formation in mice generated from such cells (Okita *et al*., 2007; Nakagawa *et al*., 2008). The scientific aim of this study is to improve techniques for the generation of iPS cells, in order to find the safest and most efficient way to de-differentiate adult mouse cells into the pluripotent state. In this study, I used a non-viral, transposon-based gene delivery method, the *Sleeping Beauty* expression system (Ivics *et al*. 1997). The advantage of this system over viral methods is that the

transposon integrates randomly at the genome level and does not show a pronounced bias for integration into genes. I were able to generate iPS lines from three different genetic backgrounds by using this technique. These lines were found to be pluripotent and differentiated into multiple lineages both *in vitro* and *in vivo*.

1.2 Objective of this study

This scientific study is aimed to generate novel information on the generation and maintenance of iPS cells from mouse fibroblasts on their differentiation towards cardiac lineage. I systematically investigated the effect of the origin of the genetic background in order to be clarified this novel technology prior to clinical progress. The iPS cells were analyzed and compared comprehensively with existing embryonic stem (ES) cell lines. In addition, practical approaches can be the basis for the researcher to improve the understanding on the reprogramming of the cells.

2. MATERIALS AND METHODS

2.1 PLASMID CONSTRUCTION

The SB transposon-based expression vector (Figure5) was constructed as follows. First, an IRES/eGFP cassette was inserted into the EcoRI/EcoRV site in the SB transposon vector pT2BH (Ivics *et al*. 1997). Second, an EF1α promoter was obtained from the pEF-GFP construct (Matsuda and Cepko 2004) (Addgene plasmid 11154) and inserted into the pT2BH-IRES/eGFP construct at the EagI/EcoRI sites. Third, the OSKM fragment from the FUW-OSKM constructs (Carey *et al*. 2009) (Addgene plasmid 20328) was cut at EcoRI sites, and then inserted into the pT2BH-IRES/eGFP construct at the EcoRI site.

2.2 CELL CULTURE

Primary mouse embryonic fibroblasts (MEFs) were prepared from 13.5 d.p.c mouse fetuses derived from three different genetic backgrounds: C57Bl6 inbred, C57Bl6xDBA/2J F1 hybrid, and ICR outbred using standard protocols (Robertson 1987). MEFs were cultured in FM medium (Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS, HyClone) and 50 U/mL penicillin, 50 μg/mL streptomycin). ES cells and iPS cells were cultured in ES medium (DMEM supplemented with 15% (v/v) FBS (Sera Laboratories International, West Sussex, RH17 5PB, UK), 1,000 U/mL mouse leukemia inhibitory factor (LIF, ESGRO, Chemicon International), 0.1 mM nonessential amino acids (NEAA, Gibco BRL, Life Technologies), 0.1 mM β-mercaptoethanol (β-ME, Gibco) and 50 U/mL penicillin, 50 μg/mL streptomycin). Mouse ES cells and iPS cells were cultured on mitomycin C-treated MEFs in serum-based ES medium or on 0.1% gelatin-coated dishes. All the cells were passaged with 0.25% trypsin, 0.1% EDTA and cultured at 37ºC in an incubator.

2.3 REPROGRAMMING OF MEFS USING SB VECTORS

MEFs were seeded onto 6-well plates at a density 5×10^5 cells/well one day prior to transfection in FM medium without antibiotics. The next day (day 0), 4 μg of pT2BH-EF1α-OSKM-IRES/eGFP expression vector and 0.4 μg of transposase (SB100X (Mates

et al. 2009)) were co-transfected using Lipofectamine2000 (Invitrogen) according to the manufacturer's instructions. On day 2, transfected MEFs were trypsinized with 0.25% trypsin, 0.1% EDTA and re-plated onto 10 cm tissue culture dishes containing mitomycin-C treated MEFs at a split ratio of 1:10 in ES medium. The medium was replaced every other day. On day 14, colonies were either analyzed for alkaline phosphatase (as stated by (Kobolak *et al*.)) and counted, or picked and further expanded for other characterization analyses.

2.4 EB FORMATION

To form EBs, the ES and three different genetic backgrounds of iPS cells were cultured on feeder cells for at least one cell passage. EBs were made by use of the hanging drop method (Rungarunlert *et al*. 2009). Briefly, on the starting day of differentiation, ES and iPS cells were trypsinized with 0.25% trypsin, 0.1% EDTA. EBs were formed in hanging drops by placing 800 cells in 20 µl of differentiation medium (ES medium without LIF) on the lid of Petri-dishes, the dish bottom was filled with PBS to prevent drying the cell droplets. On day 2, EBs were collected and placed into 10 mg/ml poly 2-hydroxyethyl methacrylate (poly 2-HEMA) treated-bacterial dishes and maintained in differentiation medium for 2 days.

2.5 *IN VITRO* DIFFERENTIATION ASSAY

For cardiac differentiation by spontaneously differentiation, individual EB was placed into a well of 24-well plate containing 0.1 % gelatin coated cover-slips on day4 after hanging drop. The differentiation medium was changed every second day. The EBs were cultured for a further 14 days and observed for beating daily under phase-contrast microscope. For neuronal differentiation, mouse pluripotent cells were induced to differentiate into neuronal lineage previously described with some modifications (Bibel *et al*., 2007) The medium was renewed every second days until day 14.

2.6 *IN VIVO* DIFFERENTIATION

Chimera production was used to examine the potential of iPS cell to differentiate in vivo. The standard method of stem cells preparation for injection has already been described previously (Nagy *et al*, 2003). Chimeras were produced by injection of 6-8 iPS cells into the perivitelline space of 8-cell stage embryos by using a laser system (Hamilton Thorne, Inc., XY Clone). Host embryos were obtained from ICR (in case of F1 and C57Bl/6 iPS cells) and C57BL/6xDBA/2J (in case of ICR iPS) mice and were collected at the 8-cell stage. Manipulated chimeric embryos were cultured in KSOM medium until the blastocyst stage and transferred into uterine horns (6-10 blastocysts in each horn of the uterus) of 2.5-day pseudo-pregnant recipients. Pregnancy was allowed to progress to term, followed by spontaneous parturition. Phenotypically coat color chimeras were naturally mated with ICR mice for testing germline transmission.

2.7 RT-PCR ANALYSIS

Total RNA was collected using the RNeasy Mini Kit (Qiagen). One μg of total RNA was reverse-transcribed using an oligo (dT) primer by SuperScript III Reverse Transcriptase (Invitrogen), and subjected to PCR. Standard PCR conditions were 94 ºC for 30 s, 55–62 ºC for 30 s, 72 ºC for 10 s; for 30–35 cycles. RT-PCR was performed using Gene Amp® PCR System 9700 (AB Applied System).

2.8 IMMUNOFLUORESCENCE STAINING

For immunofluorescence staining of the cells, cells were plated onto 0.1% gelatin-coated coverslips and fixed with 4% paraformaldehyde for 15 minutes at room temperature. Cells were permeabilized using 0.05% Triton-X100 for 10 minutes at room temperature, followed by 1% bovine serum albumin (BSA) for 1 hour at room temperature. Cells were washed with PBS and incubated with primary antibody overnight at 4°C. Primary antibodies used for this study include: Oct4 antibody (C-10, 1:100, Santa Cruz), Nanog antibody (1:20, R&D), SSEA-1 (480, 1:100, Abcam), cardiac Troponin T (1:200, Abcam), Desmin (1:200, Abcam), Nestin (Rat-401, dilution: 1:200, DSHB) and β-III Tubulin (Tuj1, dilution 1:2,000; Covance, PRB-435P). Following 3 washes with PBS,

cells were labeled with Alexa Flour®594-conjugated secondary antibodies (Invitrogen) for 1 hour at room temperature. Cells were then washed 3 times with PBS and covered with DAPI mounting medium (VectaShield, Vector Laboratories). The cells were analyzed and imaged by using a Zeiss AxioImager Z1 microscope (Carl Zeiss MicroImaging GmbH, Germany).

3. RESULTS

3.1 GENERATION OF IPS CELLS USING THE SB TRANSPOSON SYSTEM

The reprogramming factors (c-Myc, Klf4, Oct4 and Sox2) were cloned into the pT2BH *sleeping beauty* transposon plasmid under the transcriptional control of the Ef1a constitutively active promoter. In order to minimize the number of integration sites, I used a polycistronic expression cassette where the four factors were separated by 2A peptides (Carey *et al*. 2009). I linked this construct to green fluorescent protein (GFP) using an IRES sequence, which allowed us to monitor the transgene expression. As a negative control for iPS generation, I used the same transposon construct, which expressed only GFP.

The generation of ES cell lines from other inbred C57BL/6 or outbred strains (e.g. ICR) appears to be more difficult (Suzuki *et al*. 1999; Cheng *et al*. 2004; Tanimoto *et al*. 2008). In order to evaluate the effect of the genetic background on the generation of iPS cells, I used mouse embryonic fibroblasts (MEF) from three genetic backgrounds, an outbred (ICR), an inbred (C57BL/6) and an F1 hybrid (C57BL/6 x DBA/2J). I transfected MEFs with the transposon construct containing the polycistronic reprogramming cassette in the presence of SB100x hyperactive transposase (Mates *et al*. 2009). GFP expressing iPS-like colonies appeared 10-12 days after transfection. I did not observe a significant difference in the colony appearance, nor in the reprogramming efficiency among the three different genetic backgrounds. On day 14, I picked colonies which could be cultured and passaged repeatedly, resulting in the establishment of several stable cell lines.

3.2 CHARACTERIZATION OF IPS CELLS

This study characterized 6 lines in depth from each genetic background for pluripotent characteristics. To study the undifferentiated state of iPS cells lines, all the iPS cells first were scored for morphology, growth rate, GFP expression and alkaline phosphatase activity. The results from this study showed that the iPS cells formed typical ES-like colonies and exhibited positive staining for alkaline phosphatase. I were able to expand

these cells long-term (over 20 passages) and the proliferating cell colonies remained morphologically undifferentiated. These lines could be cryopreserved and recovered with high efficiency using standard techniques. I found that the GFP expression differed between the different iPS lines. In some lines, a subset of the cells did not express GFP, indicating silencing of the pluripotency cassette. The differences in the GFP expression might be due to differences in the copy number, number of integration sites or in silencing of the promoter in each clone.

I also analyzed the iPS lines for endogenously expressed pluripotency markers, such as, Nanog and SSEA1. The iPS lines showed positive nuclear staining for Nanog and the positive plasma membrane marker, SSEA-1, similar to that observed in ES cells. To further evaluate the expression of pluripotency markers, I performed RT-PCR analysis to identify if the exogenous expression of pluripotency genes (OSKM) could induce endogenous gene expression. I used primers for the endogenous sequences. I also analyzed the expression of additional pluripotency genes, such as Nanog, Rex1, Dax1, FoxD3, Fbxo15 and Eras. All the 18 examined iPS clones were found to express these endogenous pluripotency markers. Therefore, based on the morphology, growth rate and pluripotency marker expression, the derived iPS lines displayed typical characteristics of a pluripotent stem cell.

3.3 DIFFERENTIATION OF IPS CELLS

Next, I tested the *in vitro* differentiation potential of these cell lines. The classical method to induce ES cell differentiation is to allow ES cells to grow in suspension after LIF withdrawal from the culture medium and form aggregates known as embryonic bodies (EBs). I could determine that the 6 lines from each background formed EBs. However, I observed some differences in the morphology of the EBs. I did not find a significant relationship between the ES-like characteristics (ES-morphology or intensity of AP staining) and the capacity to form EBs when comparing the iPS lines. In addition, the activity of the reprogramming cassette (GFP expression) did not appear to influence the morphology of the differentiated EBs *in vitro*. In this study, I obtained at least 10 times

more beating EBs from the iPS lines than from the parental ES cells. I found a negative correlation existed between the level of GFP expression and the cardiac differentiation capacity of the cells within the genetic backgrounds. For example, in the F1 hybrid background, the iPS line containing the highest level of GFP expression (F1-D11) had the lowest beating rate (14%). In addition, two iPS lines derived from the ICR background, which had the highest GFP expression, also had the lowest beating rate (ICR-B7-42%, ICR-A1-58%). This observation, however, did not apply to iPS cells derived from the C57BL/6 background. These differences in the differentiation capacity might be an effect of the different genetic backgrounds.

I also analyzed the differentiated EBs for cardiac differentiation markers, desmin and TroponinT. The cells within the beating areas stained positive for these two cardiac markers. Figure 3B represents typical expression observed from each genetic background. Within differentiation culture period, ES and iPS cells were able to differentiate into cells expressing nestin, a specific antibody against the intermediate filament protein of NPCs. Furthermore, ES and iPS cells were also showed a few amount of post-mitotic neuronal marker Tuj-1. Interestingly, neuronal lineage-derived iPS cell show with approximately 2-3 times higher in number when compared to ES cells. These results demonstrated that mouse ES and iPS cells have ability to generate NPCs and differentiate further into neurons through EB formation in culture.

One defining feature of authentic pluripotent stem cells is their capacity to incorporate into developing embryos and transfer through the germ line. In order to evaluate the chimera formation potential of our cell lines, I picked the best iPS lines (based on their ability to differentiate) from each genetic background and injected individual cells into host blastocysts. The iPS cells from F1 hybrid and C57BL/6 backgrounds were injected into ICR blastocysts, whereas, iPS cells derived from the ICR background were injected into C57BL/6 blastocysts.

I obtained chimeric mice from the iPS lines derived from F1 hybrid and ICR mice. The Bl6-A4 line derived from C57BL/6 mice did not form chimeras. I are currently

performing subsequent chimera experiments using another line from this background. The chimeras from the F1 hybrid and ICR backgrounds were then mated to identify whether the cells could contribute to the germ-line. Two females mated with the chimeras derived from the F1 hybrid background produced offspring with black color, indicating germ-line transmission. The *in vivo* differentiation assay revealed that the F1 hybrid iPS cells had the best differentiation potential, even though the ICR lines performed the best *in vitro.* The cell lines with the least *in vitro* and *in vivo* differentiation potential were derived from the C57BL/6 background.

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4. NEW SCIENTIFIC RESULTS

1. I have generated for the first time of mouse iPS cells by non-viral, *Sleeping Beauty* transposon-mediated gene delivery, with four transcription factor (OSKM).

2. For the first time, the capabilities of SB transposon-derived mouse iPS cells to be fully reprogrammed have been proven by both *in vitro* and *in vivo*.

3. For the first time, a novel comparative *in vitro* study has been performed with iPS cells generated from mouse fibroblasts from three different genetic backgrounds: ICR (outbred), C57BL/6 (inbred) and F1 hybrid (C57BL/6 x DBA/2J).

5. DISCUSSION AND SUGGESTION

Since the first successfully generated iPS from fibroblasts that can be reprogrammed by retroviral delivery by four transcription factors (OSKM), a substantial number of alternative approaches have been developed to induce pluripotency in many kind of somatic cells. To properly assess the improvement that each of the methods provides and to give a more precise idea of their real contribution to reprogramming, it will be crucial to test them using commonly accepted standards. In addition to the use of oncogenes in reprogramming cocktails and the issue of viral integration, reprogramming itself may have an effect on a cell's genome, especially given that the process takes many weeks and is rather inefficient. Although reprogramming by using retroviral delivery is efficient and widely used, iPS cells-derived from retroviral vectors have insertional mutations and cause the tumorigenic.

In this study, I have shown that the *Sleeping Beauty* transposon system, containing the polycistronic reprogramming cassette is able to reprogram MEFs to the pluripotent state in three different genetic backgrounds, including an inbred (C57BL/6), an outbred (ICR) and an F1 hybrid (C57BL/6 x DBA/2J) strain. To achieve this, I utilized the SB-transposon system to deliver the reprogramming cassette, which were linked by self-cleaving peptide. This peptide has approximately 20-amino acid long 2A peptides from foot-and-mouth disease virus (F2A) and *Thosea asigna* virus (T2A). They work as self-cleaving signals and enable expression of several gene products from a single transcript (Szymczak *et al*. 2004), which facilitated multi-gene delivery to target cells. I also used a polycistronic expression cassette where the four reprogramming factors were separated by 2A peptides in order to generate the iPS cells and minimize the number of integration sites (Carey *et al*. 2009).

The *Sleeping Beauty* transposon system has similar advantages to other transposon-based systems such as *piggyBac* (Kaji *et al*. 2009; Woltjen *et al*. 2009). This gene delivery method is simple compared to viral systems. The SB transposon system has a very large cargo capacity. Our reprogramming construct has size around 11 kb. Unlike most other

DNA transposons, *piggyBac* has a capacity up to 10 kb (Ding *et al*. 2005). I were able to reprogram the cells by simple transfection, avoiding the preparation of the viral stocks in a biohazard facility. However, it is also have a disadvantage by this transient expression method which has a very low reprogramming efficiency (Okita *et al*. 2008; Stadtfeld *et al*. 2008c). By using the hyperactive form of the SB transposase, the frequency of the genomic integration was relatively high. It may also be possible to remove the integrated transgene following insertion, by using a mutant version the transposase, which is able to remove the transgene from the genome, but not able to re-integrate (Ivics Z unpublished). Therefore, it may be possible in the future to generate therapeutically safe, reprogramming factor-free iPS cells. These experiments are currently in progress in our laboratory.

Success in the generation of mouse ES cells highly depends on the mouse strain used. The most commonly used strain for ES cell generation is the 129/SV strain. The generation of ES cell lines from other inbred C57BL/6 or outbred strains (e.g. ICR) appears to be more difficult (Suzuki *et al*. 1999; Cheng *et al*. 2004; Tanimoto *et al*. 2008). However, our methods could be readily applied to other cell types such as keratinocytes, which has higher reprogramming efficiency than fibroblasts to generate iPS cells (Aasen *et al*. 2008; Stadtfeld *et al*. 2010a). To examine the development potential of the iPS cells derived from 3 different genetic backgrounds, all iPS cell lines showed that the endogenous pluripotency genes were switched on following reprogramming which was detected by immunostaining (Oct4, Nanog, SSEA-1) and RT-PCR (endogenous and endogenous-exogenous). From all three backgrounds, the cells were able to differentiate *in vitro* into cardiac and neuronal lineages by using embryoid bodies or monolayer chemically defined stepwise differentiation.

The most common strain of inbred mice used in research is the C57BL/6 mouse. This strain is the most commonly used background of genetically modified mouse strains and is currently the only inbred strain whose genome has been fully sequenced (Waterston *et al*. 2002). Several studies have been reported strain-dependent differences mostly in inbred mouse strains for cardiovascular function (Blizard and Welty 1971; Bendall *et al*.

2002; Hoit *et al*. 2002; Stull *et al*. 2006). Interestingly in this study, the majority of the examined iPS lines from each background had a better differentiation potential compared to the parental ES cells. This was particularly noticeable from lines derived from the outbred strain, where it is known that the generation of pluripotent stem cells is more difficult than in hybrid or in inbred strains. In addition, the iPS line derived from ICR outbred also performed the best *in vitro* cardiac differentiation. However, the iPS cells derived from ICR background could not develop *in vivo* differentiaton.

Our results show iPS cells to aggregate and differentiate in hanging drop and in suspension culture. Embryoid bodies recapitulate many aspects of cell differentiation during early mammalian embryogenesis and the cells can be terminately differentiated into various cell types belonging to the three germ layers (Keller 1995). The lack of structural organization and positional information within EBs during differentiation of the cells result in heterogeneity both within and between EBs. Interestingly, high yield of cardiac and neuronal population can be generated from iPS cells compared with ES cells. The differentiated cells also showed the positive expression of differentiation marker for cardiac (cardiac Troponin T and desmin) and neuron (nestin and Tuj-1). Most importantly, two lines from F1 and ICR backgrounds, the cells formed chimeras after blastocyst injection and one line from the F1 background transmitted to the germ line, this confirming this line to be an authentic pluripotent stem cell line. A potential limitation of the studies is that ES cells were compared with iPS cells of different genetic backgrounds which are know to affect functional (Takahashi and Yamanaka 2006; Okita *et al*. 2007) and gene expression pattern (Brambrink *et al*. 2006; Soldner *et al*. 2009) of the cells

Recent studies showed, that cell extracted from different somatic tissues can be re-programmed by different efficiency, e.g, liver cells require lower level of the reprogramming factors to achieve pluripotency (Aoi *et al*. 2008). Also iPS cells derived from different sources have different differentiation potential, e.g. differentiated into neurospheres, generated from adult tail-tip fibroblasts derived iPS cells retain more

teratoma-forming cells than iPS cells from embryonic fibroblasts (Miura *et al*. 2009). The genetic backgrounds of mice have crucial differences which implicate their use for studying different diseases (Erickson 1996). Many types of pluripotent stem cells are needed for detailed analysis of genetic diseases. Outbred lines are important for modeling human diseases, such as diabetes or neuronal diseases (Sullivan *et al*., 2007). Therefore, the generation of pluripotent stem cells from outbred strains might lead to improvement of these disease models. Here, I showed that iPS technology is suitable for reprogramming cells from different genetic backgrounds, even from backgrounds (e.g. ICR), where it is difficult to generate pluripotent ES cell lines. In the iPS-ICR background, I also observed a higher efficiency of differentiation than the parental ES cell line.

Some researchers advised that perhaps the combination of single-cell analysis and cell tracking with high-resolution time-lapse imaging might be the only way to truly understand the reprogramming events (Chan *et al*. 2009; Smith *et al*. 2010). In addition, the reprogramming approach still needs to be improving for a robust and efficient. For example, small molecules were used to improve the efficiency although they must be treated with caution as some of them can be tumorigenic (review in (Feng *et al*. 2009; Stadtfeld *et al*. 2010b)). The delivery method, reprogramming factors and cell types of tissues is also require, regardless of the presence of genomic modifications, however, many approaches have been reported with inefficiently reprogramming rate.

iPS technology opened up new possibilities in regenerative medicine. By reprogramming somatic cells to pluripotent stage and then differentiate them to specific lineages, the iPS technology allows patient specific stem cell therapy, without immunological side effects. Despite many optimistic predictions, the generation of safe and efficient cells for therapy is more difficult than expected. For generation therapeutically safe iPS cells one of the most crucial issue is the choice of gene-delivery system. To avoid the danger of malignant transformation, non-integrating (plasmid transfection, chemical inducers) or removable (transposone, excisable lentiviral) techniques are applicable, instead of the

most commonly used retroviral system. Nevertheless, another very important issue for the generation of good quality iPS cells is the source of the cells to be reprogrammed.

In summary, the study presented here shows for the first time that the *Sleeping Beauty* transposon system is suitable for reprogramming differentiated cells into pluripotent cells. It remains to be tested, however, if iPS cell clones can be removed the transgene after complete reprogramming, even they could give rise to germline chimeras. This system provides a new non-viral methodology for the generation of therapeutically safe pluripotent stem cells. The iPS cells generated in our system were able to differentiate both *in vitro* and *in vivo* even without the excision of the pluripotency cassette. Our results also show, that the iPS technology provides a new tool for the generation of pluripotent stem cells from genetic backgrounds where ES cell generation has been difficult.

6. PUBLICATIONS RELATED ES AND IPS STEM CELLS

International Publications:

- Muenthaisong, S., Ujhelly, O., Polgar Z., Varga, E., Ivics, Z., Pirity, M., Dinnyés, A. 2012. Generation of mouse induced pluripotent stem cells from various genetic backgrounds by *Sleeping Beauty* transposon mediated gene transfer. *Epub. Experimental Cell Research: 28 July 2012*
- Klincumhom, N., Pirity, M.K., Ujhelly, O., Muenthaisong, S., Rungarunlert, S., Tharasanit, T., Techakumphu, M. and Dinnyés, A. 2012. Generation of neuronal progenitor cells (NPC) and neurons derived from mouse embryonic stem (ES) and induced pluripotent (iPS) cells *in vitro. Accepted: Cellular Reprogramming*

International Publications in preparation:

- Generation of cardiomyocytes from mouse embryonic and induced pluripotent stem cells by using slow turning lateral vessel (STLV) bioreactor. Rungarunlert, S., Klincumhom, N., Ujhelly, O., Nemes, C., Muenthaisong, S., Techakumphu, M., Pirity, M.K. and Dinnyés, A.

Abstract in Peer-reviewed Journals:

- Muenthaisong, S., Ujhelly, O., Varga, E., Carstea A.C., Ivics, Z., Pirity, M., and Dinnyés, A. 2011. Generation of mouse *induced* pluripotent stem (iPS) cells from various genetic background by *Sleeping Beauty* (SB) Transposon mediated gene transfer. *Reprod.Fert.Dev.* 23 (1): 292.
- Muenthaisong, S., Ujhelly, O., Varga, E., Ivics, Z., Pirity, M. and Dinnyes, A. 2010. Generation of induced pluripotent stem cells from mouse embryonic fibroblasts by *Sleeping Beauty* transposon. Transgenic Research 19 (2): 87.
- Rungarunlert, S., Tar, K., Muenthaisong, S., Techakumphu, M., Pirity, M.K., and Dinnyés, A. 2010. Differentiation of mouse embryonic stem cells into cardiomyocytes by using slow tunning lateral vessel (STLV/Bioreactor). *Reprod.Fert.Dev.* 22 (1): 398.

International Abstract and Poster presentation:

- Muenthaisong, S., Rasmussen, MA., Hall, V., Ujhelly O., Dinnyes, A. and Hyttel, P. in vitro differentiation potential of porcine induced pluripotent stem cell-like cells derived from neural progenitor cells. *The 10th ISSCR Annual meeting*. Yokohama, Japan 2012.
- Hoffding, M., M.A. Rasmussen, V.J. Hall, S. Muenthaisong, A. Dinnyes, and P. Hyttel. Ultrastructure of porcine induced pluripotent stem cell-like colonies and derived embryoid bodies. *The 10th ISSCR Annual meeting*. Yokohama, Japan 2012.
- Dinnyes, A., C. Nemes, S. Muenthaisong, N. Klincumhom, S. Rungarunlert, E. Varga, Z. Tancos, A. Lauko, R. Tosoki, M. Jakus, Z. Polgar, S. Berzsenyi, H. Raveh-Amit, K.A. Kovacs, and A. Feher. Pluripotent stem cell-derived differentiated cells for toxicity testing and regenerative medicine. in Resolve International Meeting "Tissue Remodeling in Ageing and Disease - Emerging Insights into a Complex Pathology". 28. March 2012. Vienna, Austria
- Muenthaisong, S., Ujhelly, O., Varga, E., Carstea A.C., Ivics, Z., Pirity, M., Dinnyés, A. 2010. Generation of mouse induced pluripotent stem (iPS) cells from various genetic background by *Sleeping Beauty* (SB) Transposon mediated gene transfer. *37th Annual Conference of the IETS*, Olando, Florida, January 8-11, 2011.
- Muenthaisong, S., Ujhelly, O., Varga, E., Ivics, Z., Pirity, M., Dinnyés, A. Generation of induced pluripotent stem cells from mouse embryonic fibroblasts by *Sleeping Beauty* Transposon. 9th *Transgenic Technology Meeting (TT2010)*, Berlin, Germany, March 22-24, 2010.
- Muenthaisong, S., Ujhelly, O., Varga, E., Polgár, Zs., Ivics, Z., Pirity, M., Dinnyés, A. 2010. *Sleeping Beauty* (SB) transposon mediated gene transfer in mouse fibroblast to induced pluripotent stem (iPS) cells. *The 7th Annual Conference of Asian Reproductive Biotechnology Society*, Kuala Lumpur, Malaysia, November 8-10, 2010.
- Dinnyes, A., Ujhelly, O., Csilla, N., Muenthaisong, S., Rungalunlert, S., Klincumhom N., Varga, E., Lauko, A., Tosoki, R., Jakus, M., Polgar, Zs.,

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