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SCNT versus iPSCs: proteins and small molecules in reprogramming

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ABSTRACT Somatic cell nuclear transplantation (SCNT) and induced pluripotent stem cell (iPSC) technologies can be employed to change cell fate by reprogramming. The discoveries of SCNT and iPSCs were awarded the Nobel Prize for Physiology and Medicine in 2012, which reaffirmed the importance of cell fate plasticity. However, the low cloning efficiency of SCNT and differences between iPSCs and embryonic stem cells (ESCs) are great barriers and may be caused by incomplete or aberrant reprogramming. Additionally, the well characterized reprogramming factors Oct4, Sox2, Klf4 and c-Myc (OSKM) are not simultaneously expressed at high levels in enucleated or early embryonic oocytes, suggesting reprogramming may be different in the above two methods. Recent studies have demonstrated that small molecules and specific proteins expressed in oocytes and in early embryonic development play important roles in reprogramming by replacing transcription factors, erasing reprogramming memory and accelerating the speed and extent of reprogramming. In this review, we summarize the current state of SCNT and iPSCs technologies and discuss the latest advances in the research of proteins and small molecules affecting SCNT and iPSCs. This is an area of research in which chemical biology and proteomics are combining to facilitate improving cellular reprogramming and production of clinical grade iPSCs.

KEY WORDS: *SCNT, iPSC, early embryo, oocyte, differentiation, protein, small molecule*

Introduction

Each and every one of us developed from a totipotent egg following fertilization, and the journey of embryonic development from immature to specialized cells was previously considered to be unidirectional. However, in 1962, Gurdon (Gurdon, 1962) provided the first evidence that broke from this dogmatic view and demonstrated that specialization of cells is reversible by somatic cell nuclear transplantation (SCNT) in frogs. Since then, SCNT has been used to clone sheep (Wilmut *et al.*, 1997) and pigs (Betthauser *et al.*, 2000), and following great efforts, SCNT-derived human embryonic stem cells (ESCs) were finally generated from fetal dermal fibroblasts in 2013 (Tachibana *et al.*, 2013) and from 35- and 75-year-old males in 2014 (Chung *et al.*, 2014). Subsequently, adult somatic nuclei from newborn and adult female patients with type 1 diabetes were reprogrammed in to diploid pluripotent stem cells. This work showed the potential for therapeutic applications (Yamada *et al.*, 2014) and confirmed the suitability of SCNT for large mammals, and the research area is continuing to expand (Cibelli, 2014). However, the efficiency of acquiring healthy offspring and patient-specific stem cells using SCNT remains low, possibly due to incomplete or inappropriate reprogramming of the transferred nuclear genome (Campbell *et al.*, 2007). Oocytes and early embryos perform crucial functions in SCNT-mediated reprogramming, and extracts of oocytes (Bui *et al.*, 2012) have been used successfully to mediate somatic cell reprogramming. However, many of the cellular and macromolecular factors responsible for conferring totipotency or pluripotency to somatic cells remain unknown.

A current popular hypothesis assumes that the specific fac-

Abbreviations used in this paper: bFGF, FGF2, basic fibroblast growth factor; BMP, bone morphogenetic protein; ESCs, embryonic stem cells; GDF9, growth and differentiation factor 9; iPSCs, induced pluripotent stem cells; Lif, leukemia inhibitory factor; OSKM, reprogramming factors Oct4, Sox2, Klf4 and c-Myc; PGCs, primodial germ cells; SCF, stem cell factor; SCNT, Somatic cell nuclear transplantation; SNEL, reprogramming factors Sall4, Nanog, Esrrb, and Lin28; TLR3, toll-like receptor 3.

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TABLE 1

KEY INFORMATION ON SCNT IN DIFFERENT SPECIES

Date, Species and Author	Donor cell	Injection method	Cloning efficiency
1997 Sheep Wilmut *et al.*	mammary gland cell G0 phase	Perivitelline injection none	none
1998 Calf Cibelli *et al.*	fetal fibroblasts G1 phase	none MII stage	2.2%
1998 Mouse Wakayama T *et al.*	cumulus cell G0/G1 phase	Cytoplasmic injection MII stage	5.1%
1999 Goat Baguisi A *et al.*	fetal fibroblasts G1phase	Perivitelline injection MII / TII stage	6.5%
2000 Pig Polejaeva I A *et al.*	granulosa cell G0/G1 phase	Perivitelline MII stage	none
2002 Cat Shin *et al.*	adult fibroblasts cumulus cell none	Perivitelline injection MII stage	0.5%
2002 Rabbit Chesné *et al.*	cumulus cell none	Perivitelline injection MII stage	1.3%
2003 Horse Galli *et al.*	adult fibroblasts none	Cell fusion MII stage	0.8%
2005 Dog Lee B C *et al.*	ear skin fibroblasts none	Perivitelline injection MII stage	none
2006 Ferrets Ziyi Li *et al.*	fetal fibroblasts none	Cell fusion MII stage	none
2007 Buffalos Shi D *et al.*	fetal fibroblasts and granulosa cell G0/G1 phase	Perivitelline injection MII stage	none

Note: none represents no related information in the research. Cloning efficiency represents fetal / nuclear transfer embryos. G0/G1 represents Gap 0/Gap 1. MII represents metaphase II.

tors playing important roles in the maintenance of ESCs' identity also play pivotal roles in the induction of pluripotency in somatic cells. Takahashi *et al.,* acquired iPSCs from mouse and human somatic cells using retroviruses, and these cells have the ability to replicate indefinitely while maintaining pluripotency and the ability to differentiate into cells of all three germ layers (Takahashi and Yamanaka, 2006, Yu *et al.*, 2007). These cells circumvent the need for human embryos and can reduce or avoid immune rejection by generating stem cells from the patient's own cells. Therefore, iPSCs provide an attractive alternative to ESCs and hold great promise for disease modeling, drug selection and cell therapies in both regenerative medicine and agriculture (Li *et al.*, 2014). A year after human iPSCs were generated for the first time, various disease-specific iPSCs were produced that offer an unprecedented opportunity to recapitulate both normal and diseased human tissues *in vitro* (Park *et al.*, 2008). Owing to the capacity of proliferating indefinitely, iPSCs show a high efficiency for gene targeting (Hockemeyer *et al.*, 2009), which may have implications for treating diseases resulting from genetic defects. In 2013, using human iPSCs-derived cardiomyocytes from patients with hereditary cardiac disorders, Liang *et al.,* (Liang *et al.*, 2013) demonstrated the power of patient-specific iPSCs in drug selection and drug toxicity screening for establishing the optimum drug dosage for specific patients. However, low efficiency, genome instability and epigenetic memories of donor cells during reprogramming remain barriers to the future clinical use of iPSCs.

At present, achieving a perfect reprogramming method appears to be some way off. A recent comparison of SCNT and iPSCs technologies found that both can cause subtle molecular defects (Krupalnik and Hanna, 2014). In this review, we further explore the similarities, differences and the advantages, disadvantages of these methods. Compared to SCNT, iPSCs and early embryos may provide insight into possible perfect reprogramming approaches, and may be useful for identifying proteins and small molecules in oocytes or early embryos that may be critical for this process. To this end, we have reviewed the source and function of such proteins and small molecules reported to date, and summarized the recent advances in SCNT and iPSCs technologies. The most promising approaches for producing high quality, safe and effective pluripotent stem cells using proteins and small molecules are given particular attention.

The current state of SCNT and associated problems

SCNT is a method of creating reconstructed embryos from somatic cells and oocytes that consists of implanting a donor nucleus from a somatic cell into an enucleated oocyte. This approach has produced viable offspring of a number of species for reproductive purposes, and has also generated patient-specific stem cell lines for therapeutic purposes (Fig. 1). Since the birth of Dolly the sheep, SCNT has been used to clone cattle (Cibelli *et al.*, 1998), mice (Wakayama *et al.*, 1998), goats (Baguisi *et al.*, 1999), pigs (Polejaeva *et al.*, 2000), cats (Shin *et al.*, 2002), rabbits (Chesne *et al.*, 2002), horses (Galli *et al.*, 2003), rats (Zhou *et al.*, 2003), dogs (Lee *et al.*, 2005), and ferrets (Li *et al.*, 2006) from a range of cell types (Table 1). Human ESCs (hESCs) have also been generated using SCNT and shown to be equivalent to ESCs from embryos *in vivo* studies (Chung *et al.*, 2014, Tachibana *et al.*, 2013, Yamada *et al.*, 2014).

Despite multiple attempts, the efficiency of producing viable offspring by SCNT remains stubbornly low (Maruotti *et al.*, 2010). This may be due to incomplete or aberrant reprogramming, and the

TABLE 2

SUMMARY OF EXTRACTS FROM OOCYTES AND PLURIPOTENT CELLS MEDIATING REPROGRAMMING

Date and Authors	Extracts source and start cells types	Method of Permeable membrane	Mechanism
2003 Byrne *et al*	Xenopus laevis GV oocytes Adult mouse thymocytes and adult human blood lymphocytes	10-20 streprtolysin for 7 days at 18°C	Induce terminally differentiated mammalian cells to express Oct4
2008 Bui *et al.*	Mouse GV oocytes Mouse cumulus cells	200 ng/ml streptolysin O for 40 min at 37°C	Genomic reprogramming factors are present in the cytoplasm
2010 Cho *et al.*	Mouse embryonic stem cell-derived proteins Mouse fibroblasts	230 ng/μl streptolysin O for 50 min at 37°C	Neither by the contamination of donor ESCs nor by DNAs/RNAs from donor ESCs
2011 Miyamoto *et al.*	DJ-1 in oocyte proteins Porcine fibroblasts cells	30 μg/ml digitonin	Disturb expression of P53 pathway components
2012 Bui *et al.*	Pig GV oocytes Porcine ear skin fibroblasts	300 ng/ml streptolysin O for 50 min at 38.5°C	Induce demethylation of H3-K9 Promote Oct4-EGFP expression in SCNT-derived embryos
2014 Kong *et al.*	Pig oocytes with 1st polarbody and maternal vimentin protein Porcine ear fetal fibroblasts	none	Inhibit DNA double-strand breaks Down regulate p53 expression

Note: none represents no related information in the research.

exact molecular components influencing reprogramming in SCNT are poorly understood. Efficient reprogramming of differentiated cell nucleus can also be induced by oocyte extracts. Here, extracts from oocytes or pluripotent stem cells for successful efficient reprogramming have been shown in Table 2. These researches demonstrated that proteins and small compounds in oocyte and early embryos, not genetic materials, play vital roles in reprogramming process. But these proteins and small compounds in oocytes and early embryos still need to be defined and their potential functions and mechanisms are unclear.

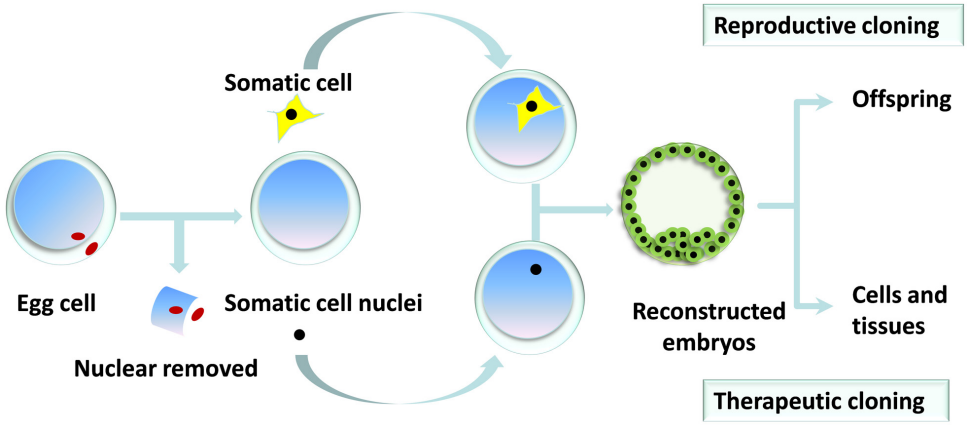

Fig. 1. Flow diagram and main purposes of somatic cell nuclear transplantation (SCNT).

The current state of iPSCs and associated problems

Through the ectopic expression of defined factors in mouse embryonic or adult fibroblasts, these differentiated cells can be reprogrammed to iPSCs (Takahashi and Yamanaka, 2006). Since iPSCs were first described, the use of these cells in the development of reprogramming technologies has been rapid, and numerous types of source cells have been employed. Researchers also use different methods to obtain iPSCs, including viral- and transgene-free methods, non-viral methods, and viral methods (Table 3). Though the last two methods are considered to take risk of genome integration, the first method is still immature, and further studies are needed for optimizing reprogramming process.

In general, these approaches up-regulate genes involved in pluripotency and self-renewal, and down-regulate lineage commitment genes (Krueger *et al.*, 2010). Current prevailing reprogramming methods affect the quality of iPSCs and great strides have been made to improve the safety, efficiency and speed of these methods. In summary, eliminating oncogenes and adding new factors such as Sall4, Tbx3 and Nr5α2 into reprogramming cocktails, adjusting existing factor combinations, and altering the proteins and small compounds present to reduce tumorigenicity have all been shown to affect the quality of the iPSCs produced (Han *et al.*, 2010, Tsubooka *et al.*, 2009, Wang *et al.*, 2013). The stoichiometry of factors and the order in which they are introduced can also affect the speed and efficiency of reprogramming (Liu *et al.*, 2013). For instance, iPSCs based on tetraploid complementation that were generated in mouse embryonic fibroblasts (MEFs) by ectopic Sall4, Nanog, Esrrb, and Lin28 (SNEL) were of higher quality than classic Oct4, Sox2, Klf4 and c-Myc (OSKM)-derived cells (Buganim *et al.*, 2014).

At present, iPSCs technologies face the problems of reprogramming extent, memory, and immunogenicity. The pluripotent genes *Fbx15*, *Oct4* and *Nanog* combined with drug-resistance genes and cloning morphologies have been used to screen positive cell lines (Takahashi and Yamanaka, 2006). At the early stages of reprogramming, iPSCs are always partially reprogrammed and possess limited differentiation potential. For example, the first iPSCs line could not produce adult chimeras and exhibited no germ transmission capacity (Okita *et al.*, 2007, Takahashi and Yamanaka, 2006). However, these partially reprogrammed cells proved powerful for investigation of reprogramming mechanisms. Meanwhile, partially reprogrammed iPSCs can elicit immune rejection (Martins-Taylor and Xu, 2012, Polo *et al.*, 2010). Although controversy concerning the immunogenicity of iPSCs remains all the time, the immunogenicity of iPSCs generated by a viral integration approach was higher than that of cells derived by an episomal method without genomic integration (Zhao *et al.*, 2011). These results suggest that iPSCs derived by viral-free and gene-free methods, such as protein- or small molecule-mediated methods, may result in lower

TABLE 3

CHARACTERISTICS, PROS AND CONS OF iPSC GENERATION METHODS

Date and authors	Delivery method	Pros	Cons
Viral methods			
2007 Okita *et al.*	Retroviral	Fairly efficient	Genome integration, possible reactivation of viral genes
2014 Zhang *et al.*	Lentiviral	Very efficient	Genome integration, possible reactivation of viral genes
2008 Stadtfeld *et al.*	Adenoviral	No genome integration	Low efficiency,
2008 Stadtfeld *et al.*	Sendai virus	No genome integration	Low efficiency, possible reactivation of viral genes
Non-viral methods			
2011 Okita *et al.*	Plasmid/DNA	Low genomic integration Non-viral, possible to carry large DNA fragments	Risk of genome integration, Low efficiency Repeated transfections
2009 Yu *et al.*	Episomal	Low genomic integration Non-viral, possible to carry large DNA fragments, no need for repeated transfections	Risk of genome integration, Low efficiency
Viral- and transgene-free methods			
2010 Warren *et al.*	mRNA	Non-viral, high efficiency, No genome integration, controllable cellular delivery	Need repeated transfections, exist immunogenic effect, expensive
2011 Miyoahi *et al.*	MicroRNA	Non-viral, fairly efficiency, no genome integration, fast	Poor repeated
2009 Kim *et al.*	Protein	No genome integration, non-viral	Low efficiency, need large amounts of pure and bioactivity proteins
2013 Hou *et al.*	Small compounds	Non-viral, high efficiency, no genome integration	Poor repeated

immunogenicity. On the other hand, the origin of the cell type can also influence the immune properties of iPSCs, possibly due to differences in reprogramming memory (Polo *et al*., 2010). The difference in transcription and epigenetic landscape between ESCs and iPSCs is that the latter type bearing some memory of their original differentiated state (Eguchi *et al*., 2014). The memory of iPSCs can result in the partial retention of the transcriptional and epigenetic features of the source cells. Additionally, iPSCs tend to differentiate into source cells when differentiation is induced. Interestingly, this phenomenon gradually disappears with multiple passages (Lee *et al*., 2014). In 2013, Kumar *et al.,* (Kumar *et al*., 2013) found that inducing the enzyme cytidine deaminase could diminish the epigenetic memory of iPSCs, resulting in a stable pluripotent state.

Proteins and small molecules in SCNT and iPSCs

In 1981, mouse ESCs (mESCs) were obtained from blastocyst pluripotent epiblast cells and cultured with inactivated fibroblast feeders, serum and other ingredients (Evans and Kaufman, 1981). It was found that cytokines from the feeder cells or serum such as leukegmia inhibitory factor (Lif) (Gardner and Brook, 1997) and bone morphogenetic protein (BMP) (Ying *et al*., 2003) were essential for maintenance of pluripotency. Approximately 20 years later, hESCs were first reported (Thomson *et al*., 1998). In apparent contrast to mESCs, Lif (Humphrey *et al*., 2004, Thomson *et al*., 1998) and BMP (Xu *et al*., 2002) have been shown to induce differentiation in hESCs. In contrast, basic fibroblast growth factor (bFGF) greatly promoted the self-renewal of hESCs (Amit *et al*., 2000). Therefore, researchers realized small molecules may cause distinct states of pluripotent in stem cells in mouse and human. In 2009, Nichols and Smith (Nichols and Smith, 2009) proposed that two phases of pluripotency can be defined: naïve and primed. The mESCs are naïve pluripotent state and lead to broad and more robust developmental potential relative to primed mouse epiblast cells and hESCs. Then, Hanna *et al.,* (Hanna *et al*., 2010) acquired naïve human ESCs/iPSCs which could grow on feeder cells in PD0325901/CHIR99021/Lif/Forskolin. After that, Ware *et al.,* (Ware *et al*., 2014) also described two routes to generate naïve hESCs by some small molecules and proteins like MEK/ERK and GSK3 inhibitors with FGF2. These studies suggest that specific proteins and small molecules play an important role in establishing and maintaining the pluripotency of ESCs among different species, and even the pluripotent state can inter-convert through different combinations of small compouds and proteins.

Small molecules can be highly influential in reprogramming procedures. During the early stages of reprogramming, activation of the toll-like receptor 3 (TLR3) innate immunity pathway was shown to be required for efficient nuclear reprogramming by comparing the viral and protein delivery of reprogramming factors (Lee *et al*., 2012). Furthermore, TLR3 activation was implemented by Poly I:C, a type of TLR3 agonist. More recently, Notch inhibition was shown to facilitate oncogene-independent generation of mouse and human iPSCs (Ichida *et al*., 2014).

One hypothesis that accounts for the enhanced reprogramming capacity posits that current strategies for inducing pluripotent stem cells lack oocyte-specific factors (Gonzalez-Munoz *et al*., 2014) such as BMPs, GDF9 (Paradis *et al*., 2009) and ASF1A (Kocabas *et al*., 2006) that are specifically enriched in oocytes,early embryos. Furthermore, these factors could also contribute to achieving more efficient reprogramming and illuminating the molecular pathways involved (Table 4). There are many more factors in oocytes and early embryos that may perform vital functions in reprogramming, many of which are untested for iPSCs generation (Table 5).

TABLE 4

PROTEINS AND SMALL MOLECULES IN OOCYTES THAT IMPROVE iPSC QUALITY AND SAFETY

Chemicals kinds	Date and Authors	Species and cell types	Methods and factors	Functions
LIF bFGF SCF	2006 Takahashi *et al.*	Mouse MEFs	Retrovirus OSKM	Give rise to iPSCs
	2007 Takahashi *et al.*	Human Fibroblasts	Retrovirus OSKM	
	2014 Kimura *et al.*	Mouse PGCs	Retrovirus OSKM	
PD0325901 CHIR99021	2008 Silva *et al.*	Mouse NSCs	Retrovirus OSKM	ERK and GSK3b inhibitors, Improve reprogramming efficiency Convert pre-iPSCs into iPSCs
	2009 Esteban *et al.*	Pig PEFs	Lentiviruses OSKM	
	2009 Lin *et al.*	Human Fibroblasts	Retrovirus OSKM	
Vitamin C	2010 Esteban *et al.*	Mouse/Human Somatic Cells	Retrovirus OSKM	Convert pre-iPSCs into iPSCs, Improve reprogramming speed and efficiency, Modulate TET1 function, Promote the demethylation of H3K36me2/me3
	2011 Wang *et al.*	Mouse MEFs	Retrovirus OSK	
	2013 Chen *et al.*	Mouse Somatic Cells	Retrovirus OSK	
BMP GDF9	2010 Samavarchi-Tehrani *et al.*	Mouse MEFs	piggyback transposition OSKM	BMP drive a MET transition, Replace some factors
	2011 Chen *et al.*	Mouse MEFs	Retrovirus Oct4	
Deng's factors	2011 Li *et al.*	Mouse Fibroblasts	Lentiviruses Oct4	Replace some factors, Improve reprogramming efficiency
	2013 Hou *et al.*	Mouse	Compounds	
GATA protein family	2015 Shu *et al.*	Mouse Somatic cells	Lentiviruses SKM	inducers for cellular reprogramming to pluripotency

Note: SCF, stem cell factor; Deng's factors are VPA, CHIR99021, 616452, Tranylcypromine, Forskolin, DZNep and TTNPB.

TABLE 5

PROTEINS AND SMALL MOLECULES IN OOCYTES AND EARLY EMBRYOS MEDIATING REPROGRAMMING

Date and authors	Species	Factors and source	Functions and mechanism
2011 Maekawa *et al.*	Mouse and Human	Glis1 Enriched in oocytes and one-cell-stage embryos	a GLI transcription factor Promote multiple pro-reprogramming pathways including Myc, Nanog, and Lin28
2011 Kei Miyamoto *et al.*	Porcine	DJ-1 Enriched in germinal vesicle stage oocytes to four-cell-stage embryos	Dimeric protein Inhibition shows perturbed expression of P53 pathway components
2013 Shinagawa *et al.*	Mouse	TH2A and TH2B Enriched in zygotes, decreases in differentiation into blastocysts	Histone variants Inducing transcriptionally active and open chromatin
2013 Yuran Song *et al.*	Porcine	CBHA none	Histone deacetylase inhibitor Increases global histone acetylation levels
2013 Kei Miyamoto *et al.*	Xenopus	Nuclear Wave1 Oocyte nucleus	One isoform of Wave enriched in brain Activation of embryonic genes in Xenopus oocytes
2014 Qingran Kong *et al.*	Porcine	Vimentin Oocyte factors	Intermediate filament Genomic protector and results in p53 down-regulation
2014 Wen *et al.*	Mouse	H3.3 Oocyte factors	Histone variants Replacement of donor nucleus-derived H3 with den ovo synthesized maternal H3.3 protein
2014 Gonzalez-Muñoz *et al.*	Human	ASF1A and GDF9 Enriched in metaphase II oocytes	Histone remodeling chaperone Acetylating H3K56, impacting the expression of core pluripotency genes

Note: none represents no related information in the research.

Advantages and strategies of using proteins and small molecules in reprogramming

Proteins and small molecules can be used to regulate biological processes including reprogramming and have distinct advantages over other approaches. Firstly, they can be synthesized in large quantities and stored for long periods until needed. Secondly, the effects are usually reversible and can often be tightly controlled by duration and concentration. Lastly, owing to their stability and easy manipulation, they are suitable for academic and industrial applications. Numerous proteins and small molecules, whether from feeder cells, serum or synthesized, have proved useful for establishing and maintaining ESCs, and many play vital roles in reprogramming, which allows genetic-free manipulation of stem cells(Li *et al*., 2014). Besides, reseachers identified small molecules can enhance genome editing in pluripotent stem cells through the CRISPR/Cas9 technology, thus the use of small molecules provides a simple and effective strategy to enhance precise genome engineering applications and facilitates the study of DNA repair mechanisms (Yu *et al*., 2015).

Hamatani *et al.,* (Hamatani *et al*., 2008) also proposed that genome-wide gene expression data could be obtained from microscopic specimens such as oocytes and pre-implantation embryos due to technological advances. Understanding the highly fluctuating dynamics of proteins and small molecules in oocytes and early embryos will be invaluable for identifying novel factors and mechanisms governing reprogramming. However, discovering and characterizing these crucial molecular determinants of reprogramming remains a challenging task.

On the one hand, the low efficiency of somatic cells cloning using SCNT is a major barrier to the successful application of this technology, and our understanding of the mechanisms underlying nuclear reprogramming in SCNT must be expanded. The use of SCNT for generating stem cells from humans and other primates has been questioned for a variety of ethical and technical reasons. Furthermore, since the discovery of iPSCs, there has emerged a large body of opposition to the use of SCNT in humans, including therapeutic cloning. In contrast, the use of iPSCs holds tremendous potential for developing cell-based therapies for degenerative diseases, drug screening, and developing disease models via production of patient-specific stem cells (Peters *et al*., 2010). In September 2014, iPSCs were trialed for the first time, on a 70-year-old Japanese woman with a debilitating eye disease (http://www.nature.com/news/japanese-woman-is-first-recipient-of-next-generation-stem-cells-1.15915). Researchers around the world have since been watching closely to see whether the cells can prevent further destruction of the retina while avoiding side effects such as inducing an immune reaction or inducing cancerous growth. Therefore, more and more such clinical trials are needed to confirm the safety of iPSCs for medical applications.

It is widely appreciated that the oocyte cytoplasmic milieu is the major factors controlling the conversion of differentiated somatic cells to pluripotent stem cells. For example, the oocyte factors BMPs, vimentin and GDF9 are known to play important roles in nuclear reprogramming and iPSCs generation. Notably, the same oocyte factors have opposite effects in different cell types or species. For instance, BMPs suppress differentiation in mouse ESCs (Ying *et al*., 2003) and promote somatic cell reprogramming in early mice embryos (Samavarchi-Tehrani *et al*., 2010), but arrest human somatic cell reprogramming (Chen *et al*., 2013b). Researchers must therefore pay particular attention when translating results between species.

Conclusion

Procedures for cellular reprogramming and for using iPSCs in clinical applications must be safe, rapid and effective, and should generate high quality pluripotent stem cells that are stable and reproducible. Reprogramming methods using SCNT and iPSCs have shown great potential. Since Dolly the sheep, SCNT has proven more popular for animal cloning experiments, although the use of methods employing iPSCs is growing. Rodriguez *et al.,* (Rodriguez *et al*., 2012) investigated signalling pathways participating in the formation of the porcine inner cell mass, and this knowledge could be applied to the development of porcine iPSCs. Perfect

reprogramming and production of high quality iPSCs remain the major targets, and expanding our knowledge of early embryonic development and ESCs differentiation will be important for achieving these aims. For instance, in 2015, Irie *et al.,* (Irie *et al.*, 2015) generated human primodial germ cells (PGCs) from ESCs and high quality naïve state iPSCs, and Sox17 is a critical specifier of human PGCs fate, it may contribute to a major ultimate goal for human health (Barrios *et al.*, 2013).

High-throughput or next-generation sequencing is continuing to improve at pace, and is making increasingly important contributions to clinical and industrial applications. For example, *de novo* sequencing, whole genomes and transcriptomes resequencing, and single cell sequencing are now possible for detecting dymanic changes in reprogramming process. These powerful technologies have the potential to greatly expand our understanding of the mechanisms underlying reprogramming, and will assist our pursuit of perfect reprogramming methods.

In summary, both SCNT and iPSCs are highly influenced by specific proteins and small molecules that are present in oocytes and early embryos. This review has attempted to emphasize the pivotal roles that these proteins and small molecules play in governing somatic cell reprogramming.

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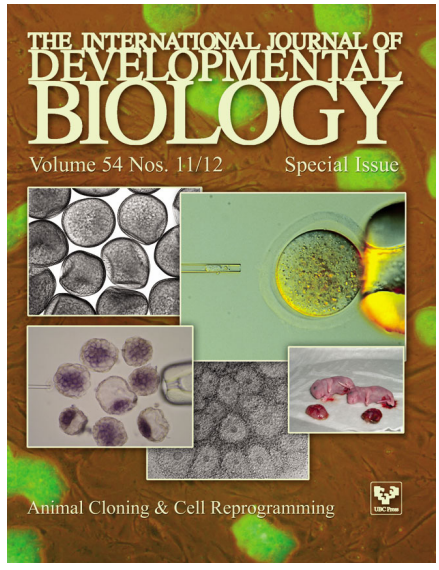

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