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28

iPS Cells: Born-Again Stem Cells for Biomedical Applications

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1. Introduction

1.1 Stemness

The fertilized egg, also known as the zygote, is a cell of total potential and plasticity and gives rise to the embryo and extra-embryonic tissues, and ultimately the whole adult organism. This property has since been termed *totipotency*, although the transition from fertilized egg to differentiated cells of the adult tissues (somatic cells) is not direct, progressing instead through lineages of successively more differentiated and committed intermediates towards the final cell type. Thus, the zygote gives rise to the trophoblast cells and inner cell mass (ICM) of the blastocyst stage embryo, the ICM gives rise to the primordial cells committed to the ectodermal, mesodermal and endodermal lineages. To give an example lineage, the ectoderm cells give rise to the neural crest stem cells, then neural stem cells, oligodendrocyte precursors, and finally oligodendrocytes which myelinate and form the white matter of the central nervous system. Each step is more specialized and less plastic than the base or 'stem' of the branch before it. Unlike the totipotent zygote, these 'stem cells' retain the ability to self-renew in addition to their plasticity. In addition to giving rise to somatic tissues during embryogenesis, the biological role of stem cells in an adult organism is to regenerate tissues lost to injury, disease or age.

1.2 History

The first stem cells discovered were the originator cells of teratomas, a rare tumor that comprised of multiple tissue types and was associated with embryonal carcinoma (EC). These EC stem cells have the then-unusual ability to self-renew indefinitely, as well as give rise to tissues from each of the three germinal layers (Kleinsmith and Pierce, 1964), a property termed *pluripotency*. Since then, many more stem cell types of varying potency have been discovered, including two more pluripotent cell types: the embryonic stem cell (ESC) is a non-cancerous analogue of the EC stem cell which is derived instead from the ICM of blastocyst stage embryos (Evans and Kaufman, 1981), and most recently induced pluripotent stem cells (iPSCs) which are produced from somatic cells that have been reprogrammed to a pluripotent state (Takahashi and Yamanaka, 2006). Pluripotent cells have since been demonstrated to have enormous potential for regenerative medicine, disease research and genetic engineering.

2. Applications of pluripotent stem cells

2.1 Animals from pluripotent cells

ESCs are the prototypical pluripotent stem cell and thus the most thoroughly characterized. They can self-renew indefinitely and are effectively immortal in cell culture. Although they lack the self-organizing capabilities of the fertilized egg, they can form any tissue in the adult organism as demonstrated by two key studies: injecting ESCs into blastocysts gives rise to chimaeric animals with tissues contributed by the injected ESCs as well as the original ICM (Moustafa and Brinster, 1972); injection of ESCs into blastocysts that have been rendered tetraploid (four genome copies, and therefore genomically incapable of forming a complete organism) produces animals wholly derived from the injected ESCs (Eggan et al., 2002). The latter technique is possible because tetraploid blastocysts retain the structural organization of a normal blastocyst, and although the tetraploid ICM will inevitably die out or senesce (and be replaced by the injected cells), the trophoblast component retains its function despite tetraploidy since trophoblasts eventually fuse and become polyploid anyway upon embryonic implantation into the maternal uterus. These properties are shared with all pluripotent cells, EC cells injected into blastocysts can also give rise to chimaeric animals (Mintz and Illmensee, 1975). Because of this potential, ESCs very quickly became a focus of applied research.

2.2 ESCs in genetic engineering and animal disease modeling

Modern reproduction techniques make it possible for a single ESC to give rise to a whole animal, greatly simplifying the process of genetically engineering animals. Previously, animals were bred extensively to isolate beneficial random mutations fertilized eggs were microinjected with DNA for random genomic integration (Gordon et al., 1980), or engineered animals were derived from nuclear-cloned somatic cells that had been engineered to the desired genotype; such a technique was used to generate cattle that lacked the prion protein and were thus made completely immune to bovine spongiform encephalopathy (BSE; mad cow disease, which transmits to humans as the variant Creutzfeldt-Jakob)(Richt et al., 2006). ESCs are easier to genetically engineer due to their infinite self-renewability, allowing a very small number of drug- or marker-selected cells to regenerate a whole culture or stable cell line. This technique has been used to generate a variety of mouse genetic models including sickle cell disease (Wu et al., 2006), thalassemia (Ciavatta et al., 1995), microcephaly (Pulvers et al., 2010), and T-cell lymphoma (Pechloff et al., 2010), as well as a p53-knockout rat for cancer research (Tong et al., 2010).

2.3 *In vitro* disease modeling using pluripotent cells

A major obstacle to disease research is the difficulty of acquiring diseased cells for study, usually because they are difficult to obtain from a living patient. For example, neurons are not easily obtained from a patient afflicted with Down syndrome, making detailed cell biology study of the neuronal basis for mental retardation impossible, and limiting our understanding of this disorder to more superficial behavioral neurological or postmortem pathological descriptions. However, a Down syndrome human ESC line as well as lines for other chromosomal trisomies have recently been derived (Biancotti et al., 2010), as has a human ESC line homozygous for Sickle Cell Disease (Pryzhkova et al., 2010). All were generated from embryos rejected by preimplantation genetic diagnosis (PGD) screens following *in vitro* fertilization (IVF). These lines allow cell culture study of diseased neurons,

or any other cell type, by differentiating diseased ESCs into any cell type of interest; however researchers are still limited by the small number of diseased human ESC lines available.

Cloned embryos can be derived from adult cells using somatic cell nuclear transfer (SCNT), a technique made famous by the cloning of the sheep Megan, Morag and Dolly in the 1990s (Wilmut et al., 1997). It has been proposed that new diseased human ESC lines can be derived using this technique to make cloned embryos from diseased patients, and then harvesting them to create novel diseased ESC lines for disease study. At the time of this writing, SCNT for this application (Therapeutic Cloning) is currently legal in the United States and the European Union, but its legal status in these states as well as elsewhere across the world has been subject to numerous prior and continuing legal challenges. Although several large organizations continue to research this technology, it has been supplanted in recent years by alternate techniques for deriving patient-specific pluripotent stem cells.

2.4 Therapeutic potential of pluripotent stem cells

Pluripotent stem cells have been studied as, and shown great potential to be, a source of cell replacement therapies in a myriad of disease and injury models. Several human ESC lines have been differentiated into high-purity cardiomyocyte cultures that improve cardiac performance when transplanted into infracted rat hearts (Caspi et al., 2007). ESCs have also been differentiated into neural precursors and neurons including dopaminergic neurons which reverse the disease progression of Parkinsonian rats (Yang et al., 2008). In a model of spinal cord injury, ESC-derived oligodendrocytes transplanted into crushed rat spinal cords successfully restored locomotive function to the animals. Pancreatic beta cells, the insulin-secreting cells whose absence causes type I diabetes mellitus, have also been derived from ESCs and cure the diabetic phenotype of the mouse streptozotocin-induced model of diabetes upon transplantation (Kim et al., 2003). These are but a choice selection of the vast amount of scientific literature detailing the regenerative potential of ESCs.

At the time of this writing, two clinical trials are underway for ESC-based regenerative therapies in humans: an evaluation of human ESC-derived oligodendrocyte precursors to rescue neurologically complete spinal cord injury conducted by Geron Corporation, and ESC-derived retinal-pigmented epithelium for treatment of macular degeneration and Stargardt's macular dystrophy, which are major causes of blindness, conducted by Advanced Cell Technology Incorporated. A third proposed clinical trial is currently in the approval process between the Food and Drug Administration and applicant California Stem Cell Incorporated for ESC-derived motor neurons as a cure for type I spinal muscular atrophy, the leading genetic cause of infant mortality. These trials represent the first step in the direct evaluation of the therapeutic potential of pluripotent stem cells in human patients.

2.5 Pitfalls and obstacles to the use of ESCs

Transplants of ESC-derived tissues and biological devices are just as subject to immune rejection as conventional organ transplants, even more so due to the limited selection of human ESC lines. Although the engineering of non-immunogenic ESCs has been the subject of many academic initiatives and company startups, ongoing clinical and preclinical research for ESC-therapies is focused, for the mean time, on immune-privileged regions of the body: specifically the brain, eye and spinal cord. A second scientific concern is the purity of ESC-derived transplants because of the hazard posed by contaminating undifferentiated

ESCs that, if transplanted, can proliferate and form teratomas. The elimination of these leftover ESCs has been approached by several strategies: purification of differentiated cells by labeling and cell sorting (Pruszak et al., 2007), the engineering of special "suicide gene"-containing ESCs (Schuldiner et al., 2003), and the treatment of cells to be transplanted with chemotherapeutics (Bieberich et al., 2004). The concomitant destruction of stem cells by anti-cancer therapies reflects the generalized similarity between stem cells and cancer cells [reviewed in (Reya et al., 2001)].

As many as seven human embryos are sacrificed for each new human ESC line derived (Thomson et al., 1998); while the ethics of this are philosophically subjective they have nonetheless given rise to numerous high-profile legal challenges to continued ESC research and funding. In addition, the patent on derivation of human ESC lines is held by the Wisconsin Alumni Research Foundation. Until its expiration in 2016, commercial users wishing to use Wisconsin ESC ("WiCell") technologies might also be required to pay a royalty.

3. Induced pluripotency

3.1 Discovery

The laboratory of Shinya Yamanaka demonstrated in 2006 that somatic cells can be reprogrammed back to a primordial phenotype functionally identical to ESCs, and termed these reprogrammed cells induced pluripotent stem cells (iPSCs)(Takahashi and Yamanaka, 2006). These iPSCs have a morphology, growth and gene expression characteristics that are indistinguishable from ESCs. Like ESCs they also form teratomas consisting of tissues from all three germ layers when injected into immunodeficient animals (Takahashi and Yamanaka, 2006), and give rise to entire animals when injected into tetraploid blastocysts (Kang et al., 2009). iPSCs also have stable telomere lengths like ESCs (Marion et al., 2008) as well as an epigenetic state reflecting reversion back to pluripotency, although traces of the donor cell's epigenetic imprint are retained in early-passage iPSCs (Kim et al., 2010).

Pluripotency is typically induced by overexpressing in somatic cells the stem cell genes Oct3/4, Sox2, cMyc and Klf4 (Takahashi and Yamanaka, 2006) (collectively termed the Yamanaka factors) or by an alternate combination of Oct3/4, Sox2, Nanog and Lin28 (Yu et al., 2007) (the Thomson factors; this repertoire has not been extensively replicated in the literature). Retroviruses or lentiviruses are the standard vectors for inserting and over-expressing these transgenes for a period of 2-3 weeks. During which the formation of early ESC-like colonies are observed [Figure 1]. These colonies stain positively for alkaline phosphatase, a marker which distinguishes undifferentiated cells from fibroblasts, and when clonally selected and propagated they express the ESC markers SSEA-1 and Oct3/4 and assume a phenotype indistinguishable from ESCs (Takahashi and Yamanaka, 2006).

3.2 Molecular mechanisms of induced pluripotency

Induced pluripotency is a remarkably successful technique, although our understanding of the underlying mechanisms are limited. The Yamanaka combination of reprogramming factors wasn't arrived at by a serendipitous leap of understanding, but instead careful and methodical experimentation. When Yamanaka sought to reprogram skin cells into ESCs, he began with a list of 24 candidate genes identified by extensive review of ESC literature. Overexpression in fibroblasts for two weeks gave rise to ESC-like colonies expressing the pluripotency marker Fbxo15. After a yearlong process of elimination, his lab was able to replicate this result with just 4 genes: Oct3/4, Sox2, cMyc and Klf4 (Takahashi and Yamanaka, 2006).

It has been known for some time that Oct3/4, Sox2 and Nanog comprise the core of the pluripotency transcriptional network, as the deficiency in either one causes ES cells to lose pluripotency (Avilion et al., 2003; Mitsui et al., 2003; Nichols et al., 1998). It is interesting to note, however, that too much Oct3/4 or Sox2 can also disrupt pluripotency. As little as a two-fold excess in either causes ESCs to differentiate (Kopp et al., 2008; Niwa et al., 2000). Oct3/4, Sox2 and Nanog all occupy each others' promoters, and more than 90% of promoters bound to by Oct3/4 and Sox2 are also occupied by Nanog (Boyer et al., 2005). Although all three are required for pluripotency, Nanog overexpression is not required for induced pluripotency. Adding Nanog to the mix, however, increases reprogramming, as does combining the Yamanaka and Thomson reprogramming repertoires (Liao et al., 2008).

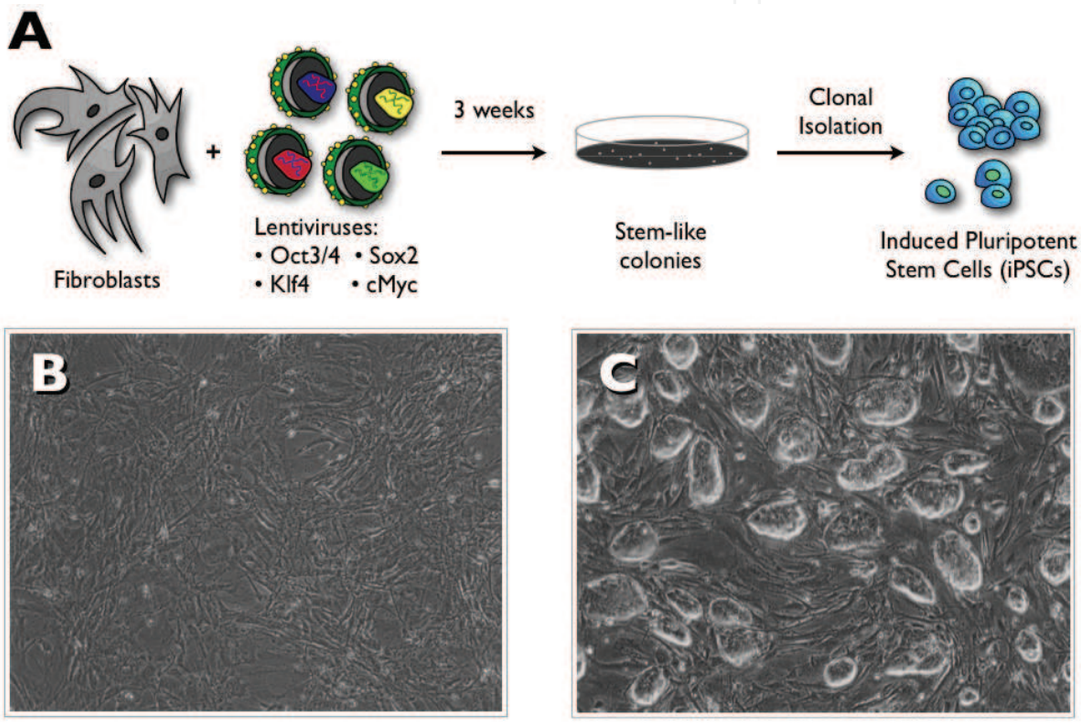

Fig. 1. Generation of iPSCs. A: Although there are a number of ways to generate iPS Cells, the model reprogramming experiment uses lentiviral vectors to integrate the Yamanaka factor transgenes into skin fibroblasts and over-express them for a period of three weeks. After this period of time, stem cell-like colonies become apparent in the reprogrammed culture which, following selection and characterization, will give rise to stable iPS Cell lines. B: Fibroblasts before reprogramming have typical morphology and grow in confluent cell monolayers. C: iPS Cells, however, grow in dense, elevated and round colonies with the characteristic "glass edge." They are microscopically indistinguishable from ESC cultures.

The use of the RNA-binding protein Lin28 as one of the Thomson factors suggested a role for microRNAs during the reprogramming process, and since then a number of pluripotency-regulating microRNAs have been identified (Zhong et al., 2010). Of particular interest is the miR-302 family of microRNAs, which induce stem cell-like plasticity when overexpressed in skin cancer cells (Lin et al., 2008).

Reprogramming to pluripotency is accompanied by the demethylation of promoter regions of known pluripotency genes, and this is observed in both iPSCs (Park et al., 2008b) and cloned embryos generated by SCNT (Lan et al., 2010). In partially or incompletely reprogrammed cells generated by either method, this demethylation is incomplete (Bourchis et al., 2001; Takahashi and Yamanaka, 2006). Likewise, chromatin alterations are also observed in reprogrammed cells as well as cloned embryos, and both of these processes are enhanced by histone deacetylase inhibitors (Han et al., 2010). Histone demethylation, particularly at the promoters of pluripotency genes, is also observed but it is not understood how this occurs during induced pluripotency.

3.3 The cancer generalization

Despite the success and reproducibility of this reprogramming technique, the permanent integration of additional copies of stem cell genes with high expression promoters poses a significant oncogenic hazard; in the earlier studies one in five chimaeric mice derived from iPSCs died from tumors resulting from spontaneous reactivation of reprogramming genes (Okita et al., 2007). Although the Yamanaka and Thomson factors are highly expressed in ESCs, they are either oncogenes themselves or associated with a poor clinical outcome when detected in cancers. cMyc specifically is one of the most well-characterized oncogenes, but Oct3/4 expression in animals also results in death due to extreme proliferation of undifferentiated progenitors (Hochedlinger et al., 2005). [Oct3/4 actually has no known role outside of pluripotent biology and when conditionally deleted in adult animals results in no detectable phenotype or defect in healing (Lengner et al., 2007)]. Oct3/4 (Gidekel et al., 2003) and Sox2 (Gangemi et al., 2009) are associated with cancer cell proliferation and tumor progression, while Klf4 has been linked to an invasive progression and metastasis in epithelial cancers (Pandya et al., 2004).

While the carcinogenic hazard introduced by genetic insertion of the Yamanaka factors led to a search for alternative reprogramming techniques, the generalization that ESCs and iPSCs biologically resemble cancer cells gave rise to a new line of thought: emulating oncogenesis to enhance reprogramming. Both SV40 Large T Antigen and TERT have been shown to enhance reprogramming when included in the Yamanaka factor repertoire (Park et al., 2008b), as has the knockout or knockdown of the tumor suppressor p53 [simultaneously discovered by 5 separate groups and reviewed in (Krizhanovsky and Lowe, 2009)]. The finding that adult stem cell populations, including hematopoietic (Eminli et al., 2009), keratinocyte (Aasen et al., 2008) and neural (Kim et al., 2008) stem cells, reprogram more easily than the more differentiated cells further down their lineages is also consistent with the understanding that adult stem cells are most prone to becoming cancerous. Although these studies contribute greatly to our understanding of induced pluripotency and stem cell biology, incorporating them into current techniques to enhance reprogramming has, until quite recently, been impossible, as doing so would greatly enhance the oncogenic hazards.

In recent years, a number of alternative techniques have emerged that induce pluripotency without genomic integration of the Yamanaka factors. Plasmid vectors have been used to induce pluripotency (Okita et al., 2008), however this technique has low reprogramming efficiency and half of the putative iPSCs generated contained some form of genomic integration. Adenoviruses also achieve reprogramming at a lowered efficiency; however a fraction of reprogrammed cells displayed karyotypic abnormalities (Stadtfeld et al., 2008).

Two other approaches favor transgene insertion followed by excision upon completion of reprogramming but also have their shortfalls: a retrotransposon vector which very rarely completely excises from the genome (Woltjen et al., 2009), and Cre-Lox recombination leaving behind residual sequences (Soldner et al., 2009). Most promising, however, are two DNA-free methods of reprogramming: direct delivery of recombinant transcription factors to the donor cells (Kim et al., 2009) and transfection with modified mRNAs encoding the Yamanaka factors (Warren et al., 2010). Although these last two methods achieve reprogramming with reduced efficiency, they circumvent the permanent oncogenic hazard presented by genomic integration of the Yamanaka factors (and any supplemental genes as well) and are most likely to be used for translational iPSC applications.

3.4 Advantages of induced pluripotency

Because they are derived from somatic cells and thus genetically autologous to the donor, iPSCs circumvent most of the obstacles, which have prevented clinical implementation of ESC technology. Moreover, being patient-specific they are not subject to immune rejection, and because induced pluripotency is an embryo-free method of deriving new pluripotent cell lines they are not subject to the funding restrictions or ethical controversies on ESC-derivation. The field of iPSC research is unlikely to see the sort of legal challenges that ESC research has, having drawn endorsements from social conservatives including Republicans in America and the Catholic Church.

iPSCs have several advantages in addition to overcoming ESC-specific obstacles. On a technique level, iPSCs are easier to derive than ESCs, and iPSC lines have already been derived from several species for which no ESC lines exist (Esteban et al., 2009; Li et al., 2009; Tomioka et al., 2010; Wu et al., 2009). This is because the optimal conditions for deriving ESCs vary across species [for example, human ESCs are maintained with basic fibroblast growth factor, while mouse ESCs are maintained with leukemia inhibitory factor], while induced pluripotency is conserved across mammals. Practically, this means induced pluripotency can facilitate easier genetic engineering of animals, as the most consistent and controlled techniques involve engineering of pluripotent stem cells. iPSC-based engineering of cattle and pigs is therefore becoming a new focus of the field [reviewed in (Telugu et al., 2010)].

Induced pluripotency has also become the key technique for deriving diseased pluripotent cells. Whereas several ESC lines modeling karyotypic abnormalities (Biancotti et al., 2010) and sickle cell disease (Pryzhkova et al., 2010) exist, derivation of new diseased ESC lines is limited to PGD-rejected embryos from *in vitro* fertilization. However, in the three years since induced pluripotency was first described in humans, iPSC lines representative of a large number of genetic diseases have been derived including amyotrophic lateral sclerosis, adenosine deaminase severe combined immunodeficiency, Shwachman-Bodian-Diamond syndrome, Gaucher disease type III, muscular dystrophies, Parkinson's disease, Huntington's disease, juvenile-onset type-1 diabetes, Down's syndrome, Lesch-Nyhan syndrome carrier, Fanconi anemia, spinal muscular atrophy, long-QT syndrome and familial dysautonomia and LEOPARD syndrome (Dimos et al., 2008; Ebert et al., 2009; Park et al., 2008a; Raya et al., 2009; Lee et al., 2009; Carvajal-Vergara et al., 2010; Moretti et al., 2010). The utility of these lines is their ability to give rise to diseased tissue *in vitro* for study as well as drug testing [Figure 2], whereas previously, research on these diseases and many others has been impeded.

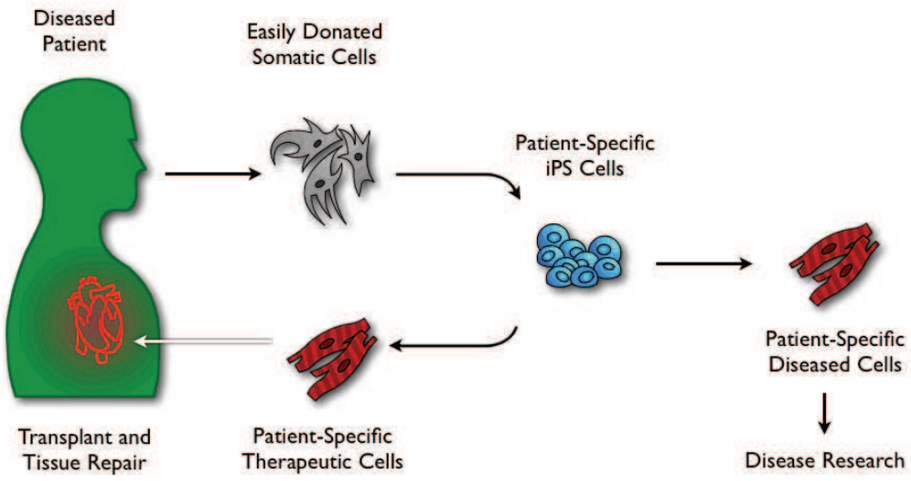

Fig. 2. Biomedical applications of iPSCs. Because iPSCs are patient-specific, neither they nor any cells derived from them are subject to transplant rejection. In this paradigm, easily obtained somatic cells such as skin fibroblasts or blood lymphocytes are reprogrammed to iPSCs, and therapeutic cells are derived from them to regenerate damaged or diseased tissue. In the case of the patient with a genetic disease, patient-specific iPSCs allow the derivation of genetically diseased tissues, which then could be subject to downstream research.

4. Reprogramming techniques

4.1 SCNT and induced pluripotency

The first experiments to demonstrate that a terminally differentiated phenotype could be reprogrammed to pluripotency took place in the mid 1990s with the generation of cloned animals, the most famous of which were Dolly the sheep and her counterparts. Megan and Morag (Wilmut et al., 1997). In these experiments, donor cell nuclei were transplanted into enucleated oocytes, a technique termed somatic cell nuclear transfer (SCNT). Upon receiving a diploid genome, the re-nucleated oocytes assumed a zygote phenotype, which formed embryos and eventually whole animals. Although these experiments were critical in showing somatic cells can be epigenetically reprogrammed to pluripotency, animals cloned by SCNT were argued to begin life not with the long telomeres typical of early-stage embryonic cells, but instead with the aged and shortened telomeres of their nuclear donors- in the case of Dolly, an adult mammary epithelial cell. However, this claim remains controversial and has been refuted by a study that used cloned cattle (Lanza et al., 2000), and a recent demonstration that mice could be continuously cloned through 15 generations without any appreciable age-related issues or cloning efficiency (Thuan et al., 2010).

Yamanaka's demonstration, ten years later, that somatic cells could be directly reprogrammed into a pluripotent state by the over-expression of exogenous Oct3/4, Sox2, cMyc and Klf4, did not suffer from telomere problems because the resulting iPSCs express telomerase at high levels, quickly extending the reprogrammed cells' telomeres to ESC-like lengths. However, introducing these transgenes using integrating retroviral vectors also brought about the risk of oncogenesis, and subsequent studies have attempted to circumvent this through one of three approaches: inserting the transgenes in a transient or

easily-excisable manner, reprogramming through the use of fewer transgenes, or reprogramming without the use of genome-integrating vectors.

4.2 Excision of reprogramming transgenes

Variations of the Yamanaka technique induce transient expression using non-integrating plasmids (Okita et al., 2008) or adenoviral vectors (Stadtfeld et al., 2008) and have produced isogenic pluripotent stem cells after many treatments, but with poor yields (in both cases, < 0.0005%). Poor reprogramming efficiency makes necessary large donor sample sizes and high multiplicities of vector transfection. Additionally, half of iPSCs reprogrammed by plasmids had genomic integration. Although the incidence of this is reduced by the use of Adenoviral vectors, karyotypic abnormalities were observed in 23% of iPSC lines produced. Nonetheless, these experiments demonstrate reprogramming without genome integration. Retrotransposon elements (Woltjen et al., 2009) and cre-lox excision (Soldner et al., 2009) have also been used to insert and subsequently remove integrated transgenes, however complete removal of transgenes occurred at only 2% efficiency in the retrotransposon-reprogrammed cells and 18% of excisions still left trace sequences in the genomic DNA. The efficiency of cre-lox excision was not demonstrated, but was consistently noted to leave proviral trace sequences.

4.3 Use of fewer transgenes

Reprogramming without using c-Myc has been demonstrated (Nakagawa et al., 2008) at 1.6% the efficiency of the standard technique of when all four genes are used. c-Myc is the most hazardous of the reprogramming factors, and its omission in this experiment reduced tumor formation in mouse chimeras past the observation period. The histone deacytelase inhibitor valproate has been shown to enhance reprogramming efficiency >100-fold (Huangfu et al., 2008a) and can substitute for the Klf4 transgene in reprogramming (Huangfu et al., 2008b), at reduced efficiency. The histone methyltransferase inhibitor BIX01294 and L-channel Ca2+ agonist BayK8644, when used together, can replace the Sox2 transgene (Shi et al., 2008) at reduced efficiency. No approach has yet replaced Oct3/4, which appears to be absolutely essential for reprogramming and is also the most reliable pluripotency marker. Reprogramming of different somatic cell types with fewer transgenes has yielded varying results. Neuronal stem cells (NSCs) have been reprogrammed to pluripotency using just Oct3/4 (Kim, 2009) and at improved efficiencies using Oct3/4 and Sox2 (Kim et al., 2008).

4.4 Direct treatment with pluripotency factors

Despite concerns with telomere length, SCNT remains the gold standard of induced pluripotency demonstrating that induced pluripotency can be accomplished through an entirely non-genetic approach (albeit with very low efficiency). This is echoed by recent experiments: somatic cells, when fused with pluripotent cells (either ESCs or iPSCs) always produce binucleate cells with both nuclei in a pluripotent state (Sumer et al., 2009). Furthermore, induced pluripotency has been demonstrated at extremely low (0.006%) efficiencies through the use of recombinant pluripotent factors (Kim et al., 2009) with multiple treatments over an 8-week period.

Permeablization of somatic cells using bacterial pore-forming toxins, and then treating them with whole cell extracts from the desired cell phenotype has shown some promise in

epigenetic reprogramming, but so far no such experiments have generated induced pluripotent stem cells. In one such study, human 293T epithelial cells treated with T cell extracts, assume a phenotype similar to T-cells, and a neuronal-like phenotype when treated with neural precursor extracts (Hakelien et al., 2002). Numerous attempts have been made to induce pluripotency by using extracts from ESCs, ECs, iPSCs and oocytes (Taranger et al., 2005; Zhu et al., 2010), however none of these have succeeded in producing stable iPSC lines.

5. Critical thinking on induced pluripotency

Although iPSCs are highly similar to ESCs in biology and function, an increasing body of literature describes defects and subtle differences between the two pluripotent cell types. Effective characterization of these phenomena is critical and will likely give rise to new and more stringent criteria by which reprogrammed cells can be evaluated for suitability.

One of the earliest characterizations of reprogrammed cells was the demonstration of DNA demethylation on the promoters of genes involved in pluripotency, such as Oct3/4 and Nanog (Okita et al., 2007; Takahashi and Yamanaka, 2006). Although these promoters exhibited near-total demethylation, indicating an activation of gene transcription, the demethylation was rarely as complete as the pattern observed in ESCs, and this was particularly true for early-passage (newly-created) iPSCs. Although these differences were small, genome-wide analysis of methylation patterns outside of these specific genes has revealed significant errors in epigenetic reprogramming (Lister et al., 2011). On a genomic scale, iPSC and ESC methylomes are similar, but most iPSC methylomes analyzed had megabase-sized loci of aberrant DNA methylation, which persist long after reprogramming and even after differentiation. Some of these loci are shared among distinct iPSC lines, suggesting that certain regions of the methylomes are susceptible to aberrant and incomplete reprogramming. There have also been reports of differences in gene expression patterns between ESCs and iPSCs, although a recent study found that most of these differences are laboratory-specific and can be attributed to microenvironment differences in growth conditions from one laboratory to the next (Newman and Cooper, 2010).

DNA sequence defects have also been described in iPSCs. Early-passage iPSCs display a range of polymorphism in copy number variant (CNV) regions compared to their parental fibroblasts (Hussein et al., 2011). As with DNA methylation, it was also found that CNVs occurred more commonly in "fragile regions" of the genome. As CNVs arise from damaged DNA improperly repaired by homologous recombination, this phenomenon suggests DNA damage and replicative stress in cells undergoing reprogramming. However, while early-passage iPSCs contain significantly more CNVs, a vast majority of these mutations put the cells at a selective disadvantage. Mid- to late-passage iPSCs therefore lose CNVs and soon approach a genomic state highly similar to ESCs. Point mutations in specific genes have also been identified in iPSCs, however unlike CNVs these display a nonrandom pattern of enrichment, with a majority occurring in proto-oncogenes and tumor suppressors (Gore et al., 2011). Half of these mutations can be traced back to the parental fibroblasts, which harbor these mutations in low frequencies; however the other half most likely arise during reprogramming and, more importantly, the subsequent selection and propagation steps. Oncogenic mutations are generalized to give a selective advantage to pluripotent cells, and although an accumulation in oncogenic mutations has also been demonstrated in ESCs, this study still establishes the need for extensive genetic testing of iPSCs before they are to be used on a clinical scale.

6. Conclusions

Although induced pluripotency as a reprogramming technique currently brings significant concerns about carcinogenicity as well as genomic and epigenomic integrity, a significant portion of the ESC research community has jumped ship in recent years in order to study iPSCs. This is because of the exciting promise these cells hold, as well as the mainstream belief that the obstacles that come with them will be overcome. With applications in a variety of fields including regenerative therapies, disease modeling, animal cloning and genetic engineering, induced pluripotency is actively transforming the stem cell community. Given how young the field is, induced pluripotency has a surprisingly well-developed body of basic research, which has already contributed enormously to our understanding on developmental biology and epigenetics, as well as given us insights on a large number of modeled genetic diseases. Taken together, the current body of literature on induced pluripotency describes why it is a very exciting time to be a part of this field.

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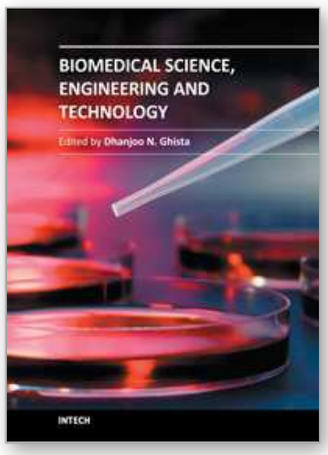

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This innovative book integrates the disciplines of biomedical science, biomedical engineering, biotechnology, physiological engineering, and hospital management technology. Herein, Biomedical science covers topics on disease pathways, models and treatment mechanisms, and the roles of red palm oil and phytomedicinal plants in reducing HIV and diabetes complications by enhancing antioxidant activity. Biomedical engineering coves topics of biomaterials (biodegradable polymers and magnetic nanomaterials), coronary stents, contact lenses, modelling of flows through tubes of varying cross-section, heart rate variability analysis of diabetic neuropathy, and EEG analysis in brain function assessment. Biotechnology covers the topics of hydrophobic interaction chromatography, protein scaffolds engineering, liposomes for construction of vaccines, induced pluripotent stem cells to fix genetic diseases by regenerative approaches, polymeric drug conjugates for improving the efficacy of anticancer drugs, and genetic modification of animals for agricultural use. Physiological engineering deals with mathematical modelling of physiological (cardiac, lung ventilation, glucose regulation) systems and formulation of indices for medical assessment (such as cardiac contractility, lung disease status, and diabetes risk). Finally, Hospital management science and technology involves the application of both biomedical engineering and industrial engineering for cost-effective operation of a hospital.

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