Malaysian J Pathol 2012; 34(1) : 1 – 13

REVIEW

Induced pluripotent stem cells in research and therapy

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Abstract

Induced pluripotent stem cells (iPSC) are derived from human somatic cells through ectopic expression of transcription factors. This landmark discovery has been considered as a major development towards patient-specific iPSC for various biomedical applications. Unlimited self renewal capacity, pluripotency and ease of accessibility to donor tissues contribute to the versatility of iPSC. The therapeutic potential of iPSC in regenerative medicine, cell-based therapy, disease modelling and drug discovery is indeed very promising. Continuous progress in iPSC technology provides clearer understanding of disease pathogenesis and ultimately new optimism in developing treatment or cure for human diseases.

Keywords: embryonic-like properties, regenerative medicine, disease modelling, patient-specific therapy, gene therapy.

INTRODUCTION

Direct reprogramming of human somatic cells to pluripotent state was achieved in 2007 through the use of four transcription factors namely Oct4, Sox2, Klf4 and c-Myc.[1,2] The resulting cells, induced pluripotent stem cells (iPSC), exhibited embryonic-like properties with unlimited self-renewal capacity and the ability to differentiate into cell types of all three germ layers. The landmark discovery of iPSC has generated great excitement in the scientific community as iPSC do not have the ethical and moral concerns surrounding embryonic stem cells (ESC).

The success of iPSC derivation has opened up a new era in research and therapy. The therapeutic potential of iPSC in regenerative medicine, cell-based therapy, disease modelling and drug discovery is highly promising. As the science progresses, new knowledge and information are acquired at an astonishing rate resulting in massive growth of iPSC-related fields. Of particular growing interest is the application of iPSC in gene therapy for treating mutation-causing diseases in humans. The progress in this area, while considered still in its infancy, has been rapid with several important proof of principle studies published in the last few years. In this review, we outline the derivation of iPSC and their potential applications in research and therapy. We also describe four important disease models as examples to further impress upon the community the therapeutic potential of iPSC in gene therapy.

1. PLURIPOTENT STEM CELLS

The interest in stem cell research first started in 1963 when Ernest A. McCulloch and James E. Till reported the presence of a group of self-renewing cells in the mouse bone marrow stroma[3] that was subsequently termed as stem cells. Stem cells are defined as cells with unlimited capacity for self-renewal without senescence and the ability to differentiate into one or more cell types *in vitro* and *in vivo*.[4] ESC are pluripotent cells derived from the inner cell mass of the pre-implantation mammalian blastocyst[5] and are potential renewable sources of all human tissues for regenerative medicine and cell-based therapy. These cells are also very valuable in developmental biology to understand the early events of human development as this is poorly studied since access to primary tissue can be difficult and ethically-charged.[6] In addition, ESC are also very useful in gene therapy and drug development for novel drug discovery and toxicity testing.

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The therapeutic efficacy of ESC has been reported for several types of neurological diseases such as spinal cord injury,[7] multiple sclerosis[8] and Parkinson's disease.[9] However, the usage of ESC is a highly controversial issue on moral, social and ethical grounds. Human embryo research, including derivation of ESC from blastocyst-stage embryo, is prohibited in some European countries such as Germany, Austria, Norway and Ireland,[10] while in other countries such as the United Kingdom, Denmark and Sweden, embryonic stem cell research is permitted by legislation but remains tightly regulated. Based on moral grounds, opposers of ESC research believe that the derivation of embryonic cells is tantamount to infanticide. This is because the process involves the destruction of a blastocyst, which is considered a viable human embryo with the potential of developing into a person. ESC produced from nuclear transfer are equally controversial because these cells can generate complete embryos and can potentially be misused for reproductive cloning. These ethical issues and moral conflicts have hindered the development of ESC as viable therapeutic tool in various clinical applications.

The recent discovery of iPSC appears to be able to circumvent problems associated with the usage of embryonic stem cells in clinical applications. iPSC are derived from any differentiated somatic cells through ectopic expression of transcription factors. The first generation of iPSC was derived from mouse fibroblasts in 2006.[11] The idea stemmed from findings that showed the ability of somatic cells to be reprogrammed either through transferring of their nuclear contents into oocyte or by fusion with ESC. This indicated that unfertilised oocyte or ESC actually contain certain factors that can confer pluripotency to the somatic cells.

Yamanaka's laboratory initially screened 24 transcription factors known to be involved in early maintenance of pluripotency in embryos and embryonic cells. Using retroviral transduction technique, these factors were transduced into mouse fibroblast and transduced colonies were selected based on G418 antibiotic resistance. Subsequently, they were able to narrow down to four transcription factors, namely Oct4, Sox2, Klf4 and c-Myc, that were able to reprogramme the mouse fibroblast into iPSC. Characterisation assays demonstrated that these cells were similar to ESC in terms of morphology, proliferation, cell marker expression and teratoma formation.

In the following year, iPSC were successfully generated from human fibroblasts using the same principles applied in the mouse model by Yamanaka's laboratory[1] as well as another research group led by James Thompson in University of Wisconsin-Madison.[2] Since then, the field of iPSC has undergone tremendous growth and many subsequent studies have tried to optimise the generation of these cells. Most notably, current techniques to generate iPSC no longer rely on using retroviruses but instead have progressed to avoid viral integration into the genome of these cells. Adenovirus, for example, has been used to deliver the four reprogramming factors to mouse hepatocytes[12] while repeated transfection with plasmids carrying the programming factors also successfully generated induced pluripotent stem cells from mouse fibroblasts.[13,14]

In fact, some research groups have reported success in generating iPSC just by using small molecules or chemicals to replace certain transcription factors for a non-DNA approach. For instance, direct reprogramming of mouse embryonic fibroblasts was successfully carried out using purified proteins of Oct4, Sox2, Klf4 and c-Myc. These proteins were first incorporated with poly-arginine peptide tags, to enable protein migration through the cell plasma membrane, and then supplemented into culture medium in the presence of histone deacetylase inhibitor for 22-28 days to generate iPSC colonies.[15] This non-DNA approach was also carried out in human newborn fibroblasts using poly-arginine based reprogramming transcription factors. In this experiment, iPSC were generated by co-culturing the fibroblasts with reprogramming transcription factor-expressing HEK293 cell extract for up to eight weeks.[16]

However, the most significant impact from the generation of iPSC in research and therapy is the accessibility of donor tissues. Unlike ESC, which are derived from embryos, iPSC can be generated from somatic tissues in a relatively simple and safe process. Somatic tissues successfully used include skin, keratinocytes and hair follicle[17], blood progenitor cells[18] and also tissues obtained from patients during surgery. In addition, cells such as leucocytes and epithelial cells from the oral mucosa are also prime candidates that are suitable for use as donor tissues for the generation of iPSC. Table 1 summarises the current reprogramming methods established and the types of cells used to date.

TABLE 1: Summary of current reprogramming methods and the types of cells used.

Reprogramming Method	Cell Type	Efficiency (%)	Genome Integration	Reference
Retroviral transduction				
• OSKM	• mouse fibroblasts	0.001 - 1	Yes	1
• OSKM+VPA	• human fibroblasts			11
• OSK	• neural stem cells			19
• OSK+VPA	• keratinocytes			20
• OS+VPA	• blood cells			
	• adipose cells			
	• liver cells			
Lentiviral transduction				
• OSKM	• human fibroblasts	0.1 -1.1	Yes	2
• OSNL	• keratinocytes			21
				22
				23
Inducible lentiviral				
• OSKM	• human fibroblasts	0.1 - 2	Yes	24
• OSKMN	• keratinocytes			25
	• blood cells			
	• melanocytes			
	• β-cells			
Adenoviral transduction				
• OSKM	• fibroblasts	~ 0.001	No	12
	• mouse hepatocytes			15
Plasmid transfection				
• OSK	• fibroblasts	~ 0.001	No	13
• OSNL				26
Excisable vector				
• loxP lentiviral	• fibroblasts	~ 0.1 - 1	No	22
• transposon				27
Non-DNA				
• Purified protein (OSKM)	• fibroblasts	~ 0.001 - 4	No	15
• Sendai virus (OSKM)	• adipose stromal cells			16
• mRNA (OSKM / OSKML +VPA)	• dermal fibroblasts			28
				29
• microRNA (miR-200c, miR-302s & miR-369s)				30
Cell fusion				
• Direct fusion with ESC	• adult thymocytes	~ 0.001	No	31
	• human fibroblasts			
Cell extract				
• Whole ESC cell extract	• human newborn fibroblasts	0.001	No	16
• ESC cell extract only	• Irradiated human fetal fibroblasts	0.0001 - 0.0002	No	32
• ESC cell extract + DNA methylation inhibitors	• Irradiated human fetal fibroblasts	0.017	No	32

* O: Oct4, S: Sox2, K: Klf4, M: c-Myc, N: NANOG, L: Lin-28 and VPA: valproic acid.

2. APPLICATIONS OF iPSC

iPSC technology has shown tremendous potential in medical and research applications due to the versatility of these reprogrammed cells. In a short span of six years after the initial iPSC generation, a broad spectrum of diseases has already been investigated using iPSC as a tool (Table 2).

2.1 Regenerative medicine

Regenerative medicine is one area undergoing significant revolution due to the advances in the field of iPSC. iPSC are highly favoured in regenerative medicine as they, like ESC, exhibit pluripotency and unlimited self-renewal capacity. Since iPSC can be derived from patients own somatic cells, they can be used in autologous cell/organ transplantation without needing to carry out extensive haplotype matching or immune suppression therapy.[33] In addition, iPSC, unlike their ESC counterparts, are less controversial without the moral, social and ethics burdens. The therapeutic potential of iPSC in regenerative medicine was demonstrated in an elegant neuronal cell replacement study for Parkinson's disease.[34] Parkinson's disease is a motor system disorder resulting from the loss of dopamine-producing brain cells. Wernig and colleagues successfully differentiated neuronal precursor cells from iPSC that were able to form neuronal and glial cell types in culture. Implantation of these cells into fetal mouse brain showed functional integration with differentiation to glia and neurons. More importantly, these cells also differentiated to dopamine neurons that improved behaviour in rat model of Parkinson's disease.

2.2 Disease modelling

Due to the versatile nature of iPSC, they are also used to model human diseases *in vitro*. Prior to iPSC, studies of genetic diseases were limited to modelling in small animals. With the discovery of iPSC, disease-specific cells are reprogrammed and differentiated to specific cell types. Interestingly, these cells are able to recapitulate the disease phenotype creating a clinical phenotype in culture dish that should theoretically contain mutations in the host tissue for establishment of *in vitro* model of the disease.[35] These models allow a more in-depth understanding of disease pathogenesis and effective drug screening for active compounds paving the way for the design of patient specific personalised medicine. So far, this has been successfully used in modelling various diseases ranging from monogenic diseases such as amyotrophic lateral sclerosis (ALS)[8], Huntington's disease[36], Hutchinson-Gilford progeria syndrome[37,38], cystic fibrosis[22] and Fragile X syndrome[39] to complex and heterogeneous diseases such as Schwachman-Bodian-Diamond syndrome[40], cardiovascular[41,42], cancer[43-45] and primary immunodeficiency[46].

2.3 Drug discovery

The process of novel drug discovery is often fraught with high cost and late attrition rate due to undetected hepatotoxicity and cardiotoxicity. For example, testing of new drugs for cardiovascular diseases was burdened with 40% of late attrition for unforeseen cardiotoxic side effects on humans that were not detected during preclinical animal model.[47] The US Food and Drug Administration in a 2004 report also estimated a potential $100 million in development costs per drug can be saved by a mere 10% improvement of drug failure prediction prior to clinical testing.[48] iPSC can be used to circumvent this problem for better drug discovery efficiency as various types of human cells can be reprogrammed for toxicology screening. In addition, iPSC, generated from a broad ethnic and genetic backgrounds, also provide a more robust and sensitive drug screening platform for more accurate prediction of toxicology and therapeutic responses in the human population.[33,49] Several studies have validated the reliability of iPSC-derived cardiomyocytes models by assessing the electrophysiologic response to well-established drugs and their effect on the QT segment.[50,51] Lee and colleagues also successfully validated the potency of candidate drugs in reversing aberrant splicing and ameliorating neuronal differentiation and migration in familial dysautonomia-derived iPSC.[52] These findings indicated the potential of iPSC technology in high throughput drug screening and further development is needed to refine the platform for more accurate and cost effective drug screening process.

3. GENE THERAPY AND iPSC

Gene therapy refers to the introduction of genetic material into particular cells or tissues for therapeutic purposes.[53] Gene therapy can be performed either by direct or indirect transfer of target gene into patients. In direct transfer, target gene is administered into patient's tissues or bloodstream after packaging with liposomes or genetically engineered vectors (viral or non-viral)

while indirect transfer requires the isolation of living cells from the patient prior to propagation and introduction of the target gene into the cells for reintroduction into the patient.[54]

As the genetic basis of most human diseases was elucidated, gene therapy was developed as therapeutic tool especially in gene correction for mutation in severe monogenic diseases. However, progress in this approach has been less than promising and hindered by many factors. One of the biggest obstacles in transcending gene therapy into clinical practice is the availability of a safe vector that is able to produce long lasting therapeutic effect with low risk of insertional mutagenesis. The first gene therapy clinical trial for X-linked SCID suffered a major setback when three out of seventeen patients developed leukaemia as a result of the activation of LMO-2 transcript in monoclonal T-cell population due to retroviral integration.[55] Even with the use of non-integrating adenovirus vector, the need of higher viral titer resulted in fatal systemic inflammatory response in ornithine transcarbamylase deficient patient following infusion.[56] Cell-based indirect gene therapy, while more complex biologically, has a few advantage when compared to direct gene therapy. The use of cells allows *in vitro* propagation prior to reintroduction into patient to achieve required therapeutic load thereby reducing the risk of potentially fatal inflammatory response. The ability of certain cells to home to specific sites also allows higher degree of specificity during reintroduction for a safer therapy.

Therefore, research is focussed on harnessing the potential therapeutic possibilities that can be achieved by combining iPSC with gene therapy for treatment of human diseases. In order to avoid similar pitfalls in direct gene therapy mentioned above, strategies have been designed to circumvent these problems. These strategies are divided into gene targeting therapy and gene augmentation therapy as illustrated in Figure 1 below.

In gene targeting therapy, spontaneous homologous recombination and zinc finger nucleases (ZFN) are the most popular approaches. Homologous recombination is an endogenous DNA repair mechanism to replace mutation permanently with short stretch of the wild type allele of the gene.[57] This approach has been used extensively in mouse ESC to investigate gene function and creation of mouse model of human diseases.[54] ZFN, on the other hand, are generated by linking a zinc finger DNA binding domain to the nuclease domain of the endonuclease Fok1 to specifically cleave a mutated gene for repair.[58] By introducing these ZFN into iPSC in the presence of a transposon carrying the correct gene sequence, the mutation can then be reversed and the phenotype corrected permanently. The use of ZFN for specific targeting for gene correction was reported to be significantly more efficient than using spontaneous homologous recombination alone.[59] Gene augmentation therapy involves providing the cells with exogenous copy of the wild type allele to correct phenotype by expressing functional proteins. This approach is suitable to be used in monogenic diseases caused by recessive mutation(s) in a single gene.

This review will now describe four important disease models using iPSC in both gene targeting therapy and gene augmentation therapy settings. For gene targeting therapy, we highlight sickle cell anemia and α1-antitrypsin (A1AT) deficiency while for gene augmentation therapy; we highlight hemophilia A and Fanconi's anemia.

3.1 Sickle cell anemia

The first landmark proof of principle study was reported in sickle cell anemia in 2007.[60] Sickle cell anemia is an autosomal recessive genetic disorder characterised by abnormal, rigid and sickle-shaped red blood cells resulting in anemia and various complications ranging from infection, chronic renal failure and generally shortened life expectancy. Previous study showed the efficacy of using lentiviral vectors expressing γ-globin in correcting sickle cell anemia in haematopoietic stem cells murine models. Long term expression, up to 10 months, was achieved without preselection in transplanted mice with erythroid specific accumulation of the antisickling protein in 52% of total haemoglobin and 99% of circulating red blood cells.[61] In this study, a humanized sickle cell anemia mouse model was first generated by replacing the mouse α-globin and β-globin genes with human α-globin and A^a and β^s genes respectively. Homozygous mice for human β^s allele displayed severe anemia consistent with sickle cell symptoms. Subsequently, tail tip fibroblasts harvested from mice with $h\beta^s/h\beta^s$ allele were used for iPSC generation using retrovirus carrying Oct4, Sox2 and Klf4 with a lentivirus encoding a 2-lox c-Myc cDNA. iPSC were then infected with adenovirus encoding Cre-recombinase to delete the lentivirus-transduced

c-Myc copies. These genetically defective iPSC were repaired by homologous recombination with a human β^A wild type globin gene. The repaired iPS cells were differentiated into haematopoietic progenitors and transplanted into humanized mutant mice after irradiation. Transplanted mice reported functional correction of sickle cell defects with marked increase in red blood counts, haemoglobin and packed cell volume levels 12 weeks post transplantation. In addition, transplanted mice also showed improved urine concentration, body weight and increased breathing. These results showed that all haematological and systemic parameters of sickle cell anemia improved substantially with the transplantation of genetically repaired haematopoietic progenitors derived from iPSC.

3.2 α1-antitrypsin (A1AT) deficiency

The use of iPSC for gene correction of α1-antitrypsin (A1AT) deficiency was recently reported.[62] Mutation in A1AT gene is most commonly associated with Pizz-associated

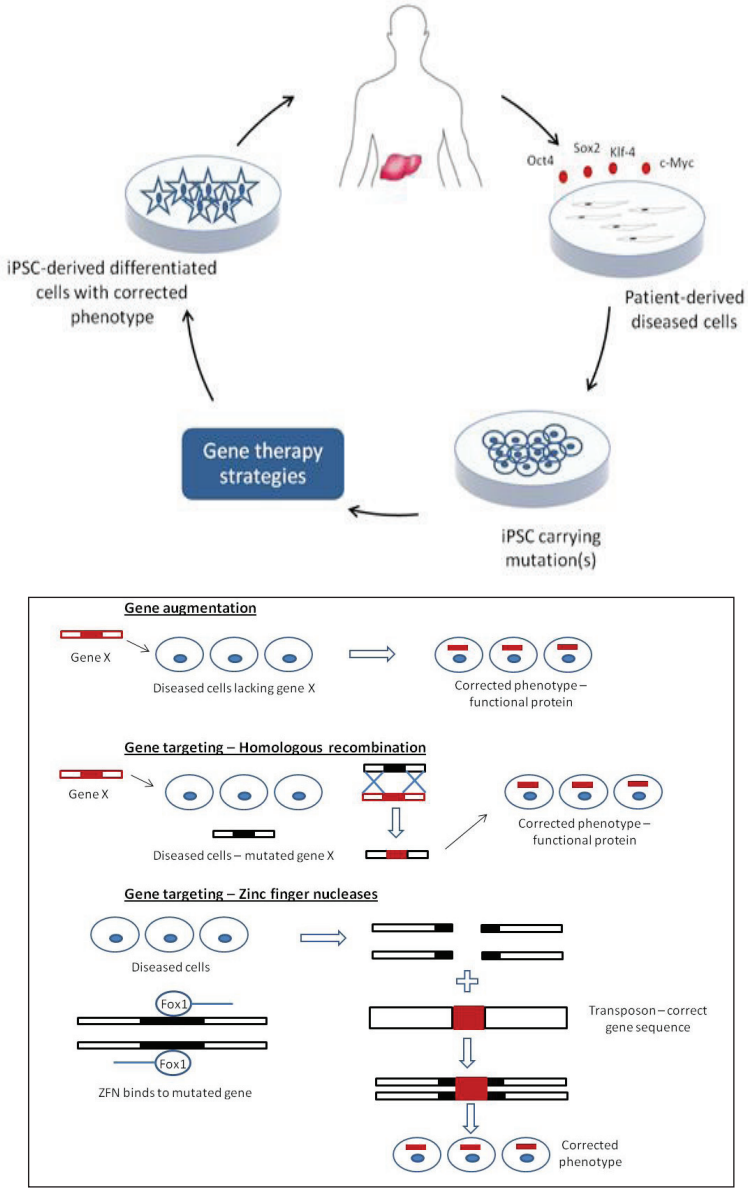

FIG. 1: Patient-derived iPSC carrying disease mutation(s) can be corrected with different gene therapy strategies and differentiated into cells with corrected phenotype for reintroduction into patient.

liver disease leading to cirrhosis. This method is different from the one used in the sickle cell anemia model as it did not require homologous recombination that will lead to residual sequences in the targeted genome. This is particularly important in order to make iPSC compatible with clinical application requirements. In this disease model, the researchers first generated iPSC from patients with Pizz-associated A1AT deficiency. Subsequently, two ZFN were designed to specifically bind upstream and downstream of A1AT mutation. The DNA flanking the mutation were then nicked by the nucleases and replaced with transposon carrying the correct sequence. Cells with corrected sequence were selected using antibiotic resistance conferred by the transposon. Once selection was completed, the transposon was removed without any genomic trace using a transiently expressed transposase. The resulting patient-derived iPSC with the corrected A1AT sequence were assessed extensively for any additional mutations prior to differentiation into hepatocytes. Transplantation of these iPSC-derived hepatocytes into immunodeficient mice was able to produce albumin and the corrected A1AT protein.

3.3 Hemophilia A

Hemophilia A is an X-linked inherited blood disorder characterised by deficiency of factor VIII protein. This is usually presented in males and could result in excessive bleeding leading to disability or death. Following the success of the sickle cell anemia model, studies were carried out to determine whether iPSC is able to correct murine hemophilia A phenotype. Hemophilia A has long been considered a prime target for gene therapy as the expression of small percentage of wild type factor VIII has significant broad therapeutic index without the need for tissue specificity.[63] However, even with a few phase I clinical trials in place, success of treating hemophilia A using gene therapy remains limited. The major obstacles are inflammatory responses to viral encoded proteins and the lack of sustained production of therapeutic gene products.[64] In the iPSC model, fibroblast from tail-tip of mice were isolated and reprogrammed to iPSC using retroviral vector carrying three transcription factors namely Oct4, Sox2 and Klf4.[65] The researchers subsequently utilised the embryoid body differentiation method to differentiate tail-tip fibroblasts into endothelial cells and endothelial progenitor cells. These endothelial/endothelial progenitor cells were injected directly into liver of irradiated hemophilia A mice. Tail clip bleeding assay, performed post transplantation, showed transplanted mice surviving up to 3 months with significant increase of plasma factor VIII level to 8% – 12% of wild type with hemophilia A phenotype correction when compared to non-transplanted mice that died within hours of the assay. This study provides another proof of principle for the potential of iPSC cell therapy in treating human monogenic disorder.

3.4 Fanconi's anemia

Fanconi's anemia (FA) is a rare genetic disease of chromosomal instability characterised by bone marrow failure, cancer pathogenesis and resistance to chemotherapy.[66] At least seven mutations in the DNA repair pathway were shown to be associated with FA namely FANCA, FANCB/D1, FANCC, FANCD2, FANCE, FANCF and FANCG.[67-69] Previous studies showed that lentiviral vectors were remarkably efficient in transducing early haematopoietic progenitors in FA knockout mice without any *ex vivo* cytokine treatment with just a single round of exposure to lentivector.[70] Even though *in vitro* and *in vivo* functional correction was demonstrated, the success of clinical trial for FA is again hampered by the issue of immunogenic responses to viral protein and the low and poor quality of haematopoietic stem cells in the bone marrow of patients for transduction. Thus, reprogramming of non-haematopoietic somatic cells to iPSC for differentiation to haematopoietic progenitors with genetically corrected FA appears promising. In the first study utilising iPSC based therapy, fibroblasts and/or keratinocytes from FA patients were isolated and genetically corrected using lentiviruses expressing FANCA and reprogrammed into iPSC using retroviral transduction with Oct4, Sox2 and Klf4.[71] Further studies are currently ongoing to evaluate the ability of genetically corrected iPSC-derived haematopoietic progenitors for FA correction in *in vivo* model.

4. CHALLENGES IN iPSC THERAPY

Results from various studies on the use of iPSC in research and therapy have been generally promising up to this point. However, there are some major challenges that must be addressed before the clinical transition of iPSC-based

therapy can happen.

The first major challenge facing iPSC clinical transition is in the reprogramming method itself. Reprogramming involves a multitude of components ranging from delivery vector(s), cocktail of reprogramming factors, growth/ differentiation factors and feeder layers for iPSC culture. At present, retrovirus is still the most efficient and commonly used vector for reprogramming. The complexity of the reprogramming method, in addition to the use of retrovirus vector, collectively increases the risk of insertional mutagenesis significantly. Insertional mutagenesis can lead to potential cancer development that could be passed through the germline to the next generation.[72] Thus, in order to make iPSC more viable as source of cells for clinical application, the complexity of the reprogramming and iPSC culture conditions must be further reduced.

Secondly, iPSC, like ESC, are predisposed to teratoma formation. Even though this predisposition is lost after differentiation[73], currently available cell purification technologies are unable to accurately separate out the differentiated cells from the undifferentiated iPSC. Transplantation of differentiated cells into patients is therefore very risky as the residual undifferentiated iPSC could lead to possible tumour formation. More studies need to be carried out to develop better purification methods before iPSC can be used clinically. At the same time, research must be actively pursued to gain more information in order to clearly delineate the differentiation pathways of iPSC into specific cell types to ensure similar function and physiology especially during *in vivo* modelling.

Recent genomic and epigenomic analyses also revealed that iPSC displayed more abnormalities than ESC or fibroblasts at the chromosomal, subchromosomal and single-base levels due to reprogramming and subsequent culture conditions.[74-76] Mutation frequency was elevated by 10 fold in iPSC than in fibroblasts[74] with chromosomal abnormalities detected earlier in iPSC culture that were not present in ESC culture[75]. In addition, iPSC epigenetic profiling revealed incomplete reprogramming with cells retaining original epigenetic markers, aberrant methylation of CG dinucleotides and abnormalities in non-CG methylation.[76] These findings showed that more extensive and high-throughput genomic and epigenomic studies must be carried out in order to fully comprehend the biological complexity in the reprogramming of somatic cells into iPSC.

TABLE 2: Summary of successful iPSC disease models.

Disease	Genetic Mutation	Method	Application	Reference
Haematological				
Sickle cell anemia	HbS	Retroviral OSK + Lentiviral M	Gene therapy	60 77
ADA SCID	ADA	Retroviral OSKM	Disease modelling	40
Hemophilia A	FVIII deficiency	Retroviral OSK	Phenotypic correction	65
Polycythaemia vera	JAK2	Retroviral OSKM	Cell differentiation: haematopoietic progenitors ($CD34^+CD35^+$)	78
Primary myelofibrosis	JAK2	Retroviral OSKM	Cell differentiation: haematopoietic progenitors ($CD34^+CD35^+$)	78
Schwachman-Bodian-Diamond syndrome	Multifactorial	Retroviral OSKM	Disease modelling	40
β-thalassemia	β-globin	Retroviral OSKM	Cell differentiation: haematopoietic cells	78
Fanconi's anemia	FA	Retroviral OSK	Gene therapy	71
Metabolic				
Lesch-Nyhan syndrome (carrier)	HPRT1	Retroviral OSKM	Disease modelling	40

Disease	Genetic Mutation	Method	Application	Reference
Gaucher's disease, type III	GBA	Retroviral OSKM	Disease modelling	40
Hyperglycemia	Multifactorial	Retroviral OSKM	Cell differentiation: β-cell-like cells	79
Glycogen storage disease 1a	G-6-P	Retroviral OSKM	Disease modelling: hepatocyte-like cells	80 81
Familial hypercholesterolaemia	LDLR	Retroviral OSKM	Disease modelling: hepatocyte-like cells	80
Crigler-Najjar syndrome	UGT1A1	Retroviral OSKM	Disease modelling: hepatocyte-like cells	80 81
Hereditary tyrosinaemia, type 1	FAHD1	Retroviral OSKM	Disease modelling: hepatocyte-like cells	80 81
α1-antitrypsin (A1AT) deficiency	A1AT	Retroviral OSKM	Gene therapy	62
Pompe disease	Knockout GAA	Retroviral OSK	Disease modelling: skeletal muscle cells	82
Wilson's disease	ATP7B	Retroviral OSKM	Disease modelling	83
Hurler syndrome	IDUA	Retroviral OSKM	Gene correction	84
Neurological				
Down's syndrome	Trisomy 21	Retroviral OSKM	Disease modelling	40
Amyotrophic lateral sclerosis (ALS)	SOD1	Retroviral OSKM	Cell differentiation: motor neurons & astrocytes	8
Spinal muscular atrophy (SMA)	SMN1	Lentiviral OSNL	Cell differentiation: motor neurons & astrocytes	85
Familial dysautonomia	IKBKAP	Lentiviral OSKM	Disease modelling & drug discovery: neural crest precursor & neurons	52
Huntington's disease	CAG repeat in Htt	Retroviral OSKM	Disease modelling: neural progenitors & striatal neurons	36
Fragile X syndrome	CGG repeat in FMR1	Retroviral OSKM	Disease modelling	39
Rett syndrome	MECP2	Retroviral OSKM	Cell differentiation: glutamatergic neuron	86
Schizophrenia	DISC1	Lentiviral OSKM	Disease modelling: neurons	87
Hutchinson-Gilford progeria syndrome	Lamin A	Retroviral OSKM	Disease modelling: neural progenitors, endothelial cells, fibroblasts, VSMC & MSC	37 38
Parkinson's disease	LRRK2 and/or SNCA	Retroviral OSKM	Cell differentiation: doperminergic neurons	34 88 89
Spinal cord injury	-	Retroviral OSKM	Regenerative therapy: neurons, astrocytes & oligodendrocytes	90

Disease	Genetic Mutation	Method	Application	Reference
Alzheimer	APP & AD	Retroviral OSKM	Disease modelling: neurons	91
Cardiovascular				
LEOPARD syndrome	PTPN11	Retroviral OSKM	Cell differentiation: cardiomyocytes	92
Timothy syndrome	CACNA1C	Retroviral OSKM	Cell differentiation: cardiomyocytes	93
LQTS, type 1	KCNQ1	Retroviral OSKM	Disease modelling: cardiomyocytes	41
LQTS, type2	KCNH2	Retroviral OSK	Disease modelling: cardiomyocytes	42
Cancer				
Chronic myeloid leukaemia	Philadelphia translocation	Retroviral OSKM	Disease modelling	43
Gastrointestinal	Multifactorial	Retroviral OSKM	Disease modelling	44
Melanoma	Multifactorial	Lentiviral Oct4	Disease modelling	45
Other				
Dyskeratosis congenita	DKC1	Retroviral OSKM	Disease modelling	94
Cystic fibrosis	CFTR	Lentiviral OSKM	Cell differentiation: lung epithelia precursor tissue	38
Duchenne muscular dystrophy	Dystrophin	Retroviral OSKM	Disease modelling	40
Primary immunodeficiencies	Multifactorial	Lentiviral OSKM	Disease modelling	46
Retinitis pigmentosa	RP9, RP1, PRPH2 or RHO	Retroviral OSKM	Disease modelling retinal progenitors, retinal pigment epithelial cells, rod photoreceptor cells	95
Dystrophic epidermolysis bullosa	COL7A1	Retroviral OSKM	Regenerative therapy: haematopoietic cells & epidermis-like keratinocytes	96

Note: This list was generated by searching peer–reviewed journals and it is by no means exhaustive as we may have missed some papers in literature.

*HbS: sickle haemoglobin, ADA: adenine deaminase, FVIII: factor VIII, JAK2: Janus kinase 2, FA: Fanconi's anemia, HPRT1: hypoxanthine phosphoribosyltransferase1, GBA: acid β-glucosidase, G-6-P: glucose-6-phosphate, LDLR: low density lipoprotein receptor, UGT1A1: UDP glucuronosyltransferase 1 family, polypeptide A1, FAHD1: fumarylacetoacetate hydrolase, A1AT: α1-antitrypsin, GAA: acid α-glucosidase, ATP7B: ATPase, Cu2+ transporting, β-polypeptide, IDUA: α-L-iduronidase, SOD1: superoxide dismutase 1, SMN1: survival of motor neuron 1, IKBKAP: I-א-B kinase complex-associated protein, Htt: Huntingtin, FMR1: fragile X mental retardation 1, MECP2: methyl CpG binding protein 2, DISC1: disrupted in schizophrenia 1, LRRK2: leucin-rich repeat kinase 2, SNCA: synuclein alpha, APP: amyloid precursor protein, AD: Alzheimer's disease, PTPN11: protein tyrosine phosphatase, non-receptor type 11, CACNA1C: calcium channel, voltage-dependent, L type, α 1C subunit, KCNQ1: potassium channel protein, KCNH2: potassium ion channel, DKC1: dyskerin, CFTR: cystic fibrosis transmembrane conductance regulator, RP9: retinitis pigmentosa 9, RP1: retinitis pigmentosa 1: PRPH2: peripherin-2, RHO: rhodopsin, COL7A1: α1-chain of type VII collagen.

CONCLUDING REMARKS

In conclusion, the discovery of iPSC is a remarkable boost to research and therapy. With less ethical restrictions and readily accessible donor tissues, more research opportunities, both basic and translational, can be carried out to better understand the nature of iPSC. The information obtained will help in elucidating the reprogramming mechanism(s) for better control to ensure the safety and efficacy of iPSC for clinical applications. The continuous progress in this technology will provide a clearer understanding of disease pathogenesis to develop treatment or cure for human diseases.

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