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REVIEW ARTICLE

Research on neurodegenerative diseases using induced pluripotent stem cells

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Abstract

Induced pluripotent stem cells (iPSC) are derived from somatic cells. These somatic cells have had their gene expression experimentally reprogrammed to an embryonic stem cell-like pluripotent state, gaining the capacity to differentiate various cell types in the three embryonic germ layers. Thus, iPSC technology makes it possible to obtain neuronal cells from any human cells. iPSC can be generated from various kinds of somatic cells and from patients with neurodegenerative diseases. Disease modelling using iPSC technology would elucidate the pathogenesis of such diseases and contribute to related drug discoveries. In this review, we discuss the recent advances in iPSC technology as well as its potential applications.

GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS (IPSC)

iPSC are stem cells derived from somatic cells. These somatic cells have had their gene expression reprogrammed to an embryonic stem cell (ESC)-like pluripotent state by the induction of certain factors expressed in ESC. iPSC were first generated in 2006 from mouse fibroblasts,[1] which was followed by human iPSC in 2007.[2] iPSC have features similar to ESC with respect to morphology, surface antigens, gene expression profiles and differentiation potential. iPSC technology makes it possible to obtain neuronal cells from living patients with various diseases. iPSC technology promise to provide important tools for the study of human disease and the development of its treatment (Fig. 1).

TRANSCRIPTION FACTORS IN REPROGRAMMING

Representative transcription factors such as octamer-binding transcription factor 3/4 (Oct3/4), sex determining region Y-box 2 (Sox2), Krüpel-like factor 4 (Klf4) and myelocytomatosis oncogene (c-Myc) are related to the reprogramming of somatic cells. They have been found to maintain stemness in ESC. Oct3/4 appears to be irreplaceable and is the determinant of direct reprogramming. Oct3/4 modulates gene expression by binding to an 8-bp consensus sequence (ATGCAAAT) in the regulatory region of various genes involved in self-renewal and differentiation.[3] Oct3/4 is expressed in the inner cell mass, and embryos deficient in Oct3/4 do not grow beyond the blastocyst stage and lack pluripotent cells in their inner cell mass.[4] Sox2, Klf4 and c-Myc are expressed in multiple adult tissues and can be replaced by other orthologues during reprogramming into iPSC.[5] Sox2 is a DNA-binding protein and interacts with a specific DNA sequence on gene enhancers.[6] Sox2 interacts with Oct3/4 to make a stable DNA binding. Sox2 is also expressed in the inner cell mass of embryos, and the Sox2-deficient mutant fails to develop into an epiblast.[7] Klf4 is a zinc-finger transcription factor that binds the core sequence (CACCC) and regulates cell proliferation and differentiation.[8] Klf4 also has a role in the inactivation of the p53 tumour suppressor gene that represses NANOG, indirectly supporting the expression of NANOG.[9] c-Myc is a proto-oncogene required for cell proliferation, and it enhances the

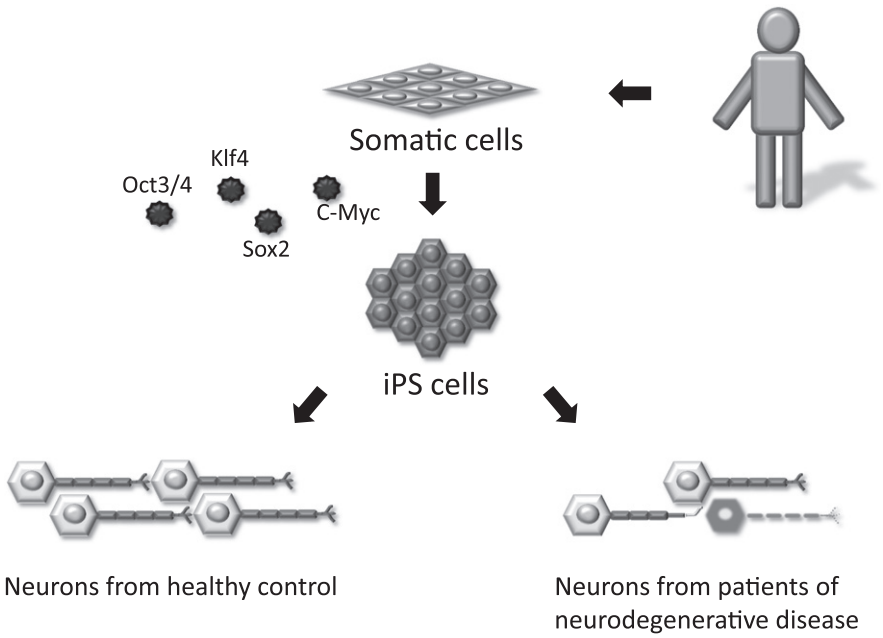

Figure 1 Modelling of neurodegenerative diseases using patient-specific induced pluripotent stem (iPS) cells. c-Myc, myelocytomatosis oncogene; Klf4, Krüpel-like factor 4; Oct3/4, octamer-binding transcription factor 3/4; Sox2, sex determining region Y-box 2.

efficacy and speed of the process in iPSC induction.[10] c-Myc occupies the promoter that interacts with the histone acetyltransferase complex and histone deacetylase complex, and induces changes in histone acetylation patterns.[11,12] It has been postulated that chromatin modification by c-Myc provides easier access to the reprogramming transcription factors. The effect of c-Myc can be partly compensated by a histone deacetylase inhibitor, including valproic acid.[13]

Oct3/4, Sox2, and Klf4 often co-bind to the promoter region. Although the detailed mechanism is still mostly unknown, it has been suggested that genes encoding somatic cell regulators might be repressed by the binding of these factors, while self-renewal and pluripotency genes might be turned on by the binding of these factors.[14]

REPROGRAMMING METHODS

The original method for generating iPSC uses retrovirus vectors.[1] Retrovirus integrates the host genome. c-Myc, known as a proto-oncogene, could induce tumour formation.[15] As a result, several methods for improving safety have been reported. For example, omitting c-Myc from the reprogramming factors, removing the integrated factors after establishing iPSC, and the transient expression of the reprogramming factors using adenovirus vectors and plasmids have been tried. The current methods for iPSC generation can be divided into three categories based on the vector types: (i) virus (retrovirus, lentivirus, adenovirus, sendaivirus); (ii) DNA (plasmid, episomal plasmid, transposon); and (iii) cell-penetrating peptide.[16] Retroviral and lentiviral vectors have high efficacy of reprogramming despite a high risk of tumour formation. The transient expression by adenovirus vectors and plasmids shows low reprogramming efficacy, but it could avoid genomic integration.

SOURCE OF IPSC

The most common source of human iPSC is currently skin fibroblasts. iPSC are also generated from other types of cells such as human blood and human keratinocytes.[17,18] Although iPSC from these cells have been shown to express genes for pluripotency and to differentiate to three germ layers, a recent study suggests that there may be substantial molecular differences between iPSC derived from distinctive cell types, including transcriptional and epigenetic patterns and functional differences.[19] To generate human iPSC, especially a disease-specific iPSC, the burden for the cell donor must be reduced. Further technological advances are expected to optimize the reprogramming from different cell types obtained from donors and make it easier.

NEURONAL DIFFERENTIATION OF IPSC

The modelling of neurodegenerative diseases requires the differentiation of iPSC into specific neuronal cell types. The first step is the differentiation of iPSC into neuronal progenitors. Efficient neural induction of iPSC can be achieved by inhibiting activin, Nodal, TGF-β, and bone morphogenetic protein (BMP) signalling with SMAD signalling inhibitors including noggin, SB431542, and dorsomorphin.[20,21]

Acquired neuronal progenitors could be differentiated along the rostro-caudal and dorso-ventral axes using specific morphogens and growth factors. Sonic hedgehog (Shh) and BMP influence the cell fate of the dorso-ventral axis,[22] whereas fibroblast growth factor (FGF)2, FGF8, retinoic acid (RA) and Wnt1 were shown to change the cell fate along the rostral-caudal axis.[23]

Without these factors, telencephalic progenitors that express the markers of the anterior neuroectoderm are presented. Neuronal progenitors treated with cyclopamine, a specific antagonist of Shh, expressed vesicular glutamate transporter 1 and vesicular glutamate transporter 2, markers of pyramidal neurons of the cerebral cortex.[24] Basal forebrain cholinergic neurons are differentiated by FGF8, Shh and BMP9, and Shh and FGF8 are necessary for patterning of the forebrain. BMP9 induces the expression of LIM homeobox protein 8 and gastrulation brain homeobox, which are necessary for differentiation to basal forebrain cholinergic neurons.[25] Midbrain dopaminergic neurons are generated by the combination of Shh and FGF8.[26] Neuronal progenitors are influenced by Shh in the ventral direction and by FGF8 in the caudal direction.

Spinal motor neurons are produced using two developmentally relevant morphogens, RA and Shh. RA induces neuralization and caudalization, while Shh induces ventralization and converts spinal progenitor cells to motor neurons.[27] Motor neurons in the ventral regions are subdivided into two main longitudinal columns, a median motor column and a lateral motor column. Motor neurons in the median motor column send axons to axial and body wall muscles, whereas motor neurons of the lateral motor column project to ventral and dorsal limb muscles. The lateral motor column is only present in the brachial and lumbar region, where the forelimbs and hindlimbs develop.[28] The combination of RA and Shh produces the median motor column and the lateral motor column.

Cerebellar granule cell precursors are produced from ESC by the combination of rostral central nervous system induction by serum-free culture of embryoid body-like aggregates and subsequent BMP4/Wnt3a treatment. The dorsalizing effect of BMP4 likely explains the enhancing effects on the induction of cerebellar granule cells. Wnt signalling is implicated in the caudalization, although the exact role of Wnt3 in granule cell induction is not yet well understood.[29]

The peripheral nervous system, including peripheral neurons and Schwann cells, is generated via neural crest stem cells. Neural crest cells are induced from ESC or iPSC by noggin, FGF8, and Shh in co-culture with murine stromal cell line cells or noggin, and by SB431542 in feeder-free culture. A serum-free condition with some neurotrophic factors, including brain-derived neurotrophic factor, nerve growth factor, glial cell line-derived neurotrophic factor and ciliary neurotrophic factor, allows differentiation of neural crest cells into sensory and autonomic neurons and Schwann cells.[30]

DISEASE-SPECIFIC IPSC AND DISEASE MODELLING

Disease-specific iPSC were generated from neurological diseases including spinal muscular atrophy (SMA) and familial dysautonomia (FA).[31,32] SMA is an autosomal recessive disease characterized by the selective loss of lower motor neurons and progressive muscular atrophy. It is mostly caused by a homozygous loss of the survival of motor neuron (*SMN*)-1 gene, and inefficient inclusion of exon 7 in transcripts from the nearly identical *SMN2* gene results in SMN decrease.[31] The motor neurons differentiated from iPSC derived from SMA patients decreased in number and presented smaller soma size, and synapse formation appeared to be compromised. Treatment with tobramycin or valproic acid increased the SMN protein expression in iPSC from an SMA patient via increased full-length SMA mRNA by upregulation of the SMN2 promoter activity.[31,33]

FA, known as hereditary sensory and autonomic neuropathy type III, is an autosomal recessive disorder caused by mutations in the I-κB kinase-associated protein (IKBKAP) gene. FA presents dysfunction of both peripheral sensory and autonomic neurons. IKBKAP expression was reduced in neural crest precursor cells in iPSC derived from FA patients.

A decrease in neuronal class III β-tubulin-positive neuronal cells and their functional deficits were also observed. Kinetin, which has been reported to increase wild-type IKBKAP expression in patient lymphoblastoid cells,[34] reduced the levels of mutant IKBKAP splice variant and increased the level of normal IKBKAP. Kinetin also increased the number of neural crest precursor cells.[32]

iPSC have also been established in common neurodegenerative diseases including Parkinson's disease (PD) and amyotrophic lateral sclerosis. PD is the most prevalent neurodegenerative disease characterized by the loss of dopaminergic neurons. When iPSC were derived from PD patients carrying a point mutation in leucine-rich repeat kinase 2, dopamine neurons that differentiated from these iPSC expressed increased levels of α-synuclein and were vulnerable to oxidative stress.[35] A study with iPSC from PD patients with mutations in mitochondrial protein PTEN-induced putative kinase 1 demonstrated a decrease in the mitochondrial recruitment of Parkin.[36]

Amyotrophic lateral sclerosis (ALS) is characterized by rapid and progressive degeneration of motor neurons in the spinal cord and motor cortex. iPSC generated from ALS patients with a mutation in superoxide dismutase 1 and vamp-associated protein B/C have been reported.[37,38] The motor neuron-enriched culture from iPSC with the vamp-associated protein B/C mutation showed reduced levels of vamp-associated protein B/C.

The iPSC described herein partially recapitulated the phenotypes of neurodegenerative diseases. Research using human iPSC may allow the elucidation of the development and progression mechanisms of neurodegenerative diseases, leading to the discovery of new drug therapies.

DIRECT CONVERSION FROM SKIN FIBROBLASTS TO NEURONAL CELLS

One of the limitations of human iPSC technology is that generating neurons from iPSC is time-intensive. The generation of iPSC and the subsequent differentiation to neuronal cells require 1–2 months each. Recently, the direct conversion from human skin fibroblasts to neuronal cells was reported.[39,40] The transduction of three or four transcription regulators produced neuronal cells, termed induced neuronal cells, circumventing the production of pluripotent cells, within about 10 days. The technology to generate induced neuronal cells may make up for the limitation of this technology. By combining the advantages of this technology, the methods to obtain neuronal cells efficiently may be optimized.

CONCLUSIONS

We described iPSC and their application in the research of neurodegenerative diseases. Further progress in this research would contribute to the unravelling of their pathogenesis and thereby aid in the discovery of new drugs for these diseases.

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