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The Use of Induced Pluripotent Stem Cells in Drug Development

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Induced pluripotent stem cell (iPSC) technology is revolutionizing medical science, allowing the exploration of disease mechanisms and novel therapeutic molecular targets, and offering opportunities for drug discovery and proof-of-concept studies in drug development. This review focuses on the recent advancements in iPSC technology including disease modeling and control setting in its analytical paradigm. We describe how iPSC technology is integrated into existing paradigms of drug development and discuss the potential of iPSC technology in personalized medicine.

The ability of cells to differentiate into various cell types—known as "pluripotency"—is a hallmark of embryonic stem cells (ESCs). Stem cells belong to one of two major categories according to their potency of differentiation: organ-specific stem cells and pluripotent stem cells. Organ-specific stem cells generally have limited potential for growth and differentiation. In contrast, pluripotent stem cells, such as ESCs[1–3] and induced pluripotent stem cells (iPSCs),[4–6] replicate in culture dishes and are theoretically capable of giving rise to any of the cell types found in the body (**Figure 1**).

The development of cellular reprogramming techniques leading to iPSCs has dramatically changed the landscape of stem cell research and application by providing a modality that circumvents the two major issues hampering fulfillment of the great potential of human ESCs.[4–6] One is the ethical issue associated with the derivation of human ESCs from human fertilized eggs, and the other is the immunological incompatibility between ESC-derived donor organs or cells and the recipients because of histocompatibility–antigenic factors.[4–6] As iPSCs are transforming the field of regenerative medicine, the reprogramming approach is also becoming a platform for drug discovery research.

DISCOVERY OF iPSCs

Reprogramming inducers

Transduction of four genes encoding transcription factors highly functional in ESCs (i.e., Oct3/4, Sox2, Klf4, and c-Myc) was discovered to be sufficient to trigger reprogramming of both mouse and human somatic cells and to generate cells closely resembling the respective ESCs.[4–6] The term coined for these reprogrammed ESC-like cells was "iPSCs."[4] Subsequent research from our laboratory as well as from others has revealed several alternative methods for generating iPSCs.[7–9]

Among the quartet of transcription factors involved in reprogramming,[9] Oct3/4 is expressed specifically in ESCs and germ cells but not in somatic cells.[9] The forced expression of Oct3/4 in mouse or human Sox2-expressing neural stem cells can give rise to iPSCs, albeit with low reprogramming efficiency.[9] There are reports of iPSC generation even in the absence of the Oct3/4 transgene, but the efficiency of generation is very low.

Sox2, which is a key partner of Oct3/4, is expressed almost exclusively in ESCs, germ cells, and nerve cells. The deletion of Sox2 causes the death of the embryo, suggesting its crucial role in embryogenesis.[9] Sox family proteins, including Sox2, show functional overlap with each other. Although the conventional reprogramming method requires Sox2 transgene, inhibition of the transforming growth factor beta (TGF-β) was shown to be capable of replacing Sox2 in reprogramming mouse embryonic fibroblasts.[9] Moreover, in some cell types, such as neural stem cells, melanocytes, and melanoma cells, the Sox 2 transgene is not necessarily a requirement for iPSC generation.[9] These findings indicate the opportunistic nature of Sox transgene requirement in iPSC reprogramming.

Kruppel-like transcription factor 4 (Klf4) is a downstream target gene of the signaling pathway of the cytokine leukemia inhibitory factor—Stat3. Klf4 has overlapping functions with other Klf transcriptional factors (Klf2 and Klf5).[10] During the reprogramming process, Klf4 binds to the Oct3/4-Sox2 complex[11] and, together with homeobox protein PBX1, it underpins iPSC identity by regulating expression of Nanog, one of

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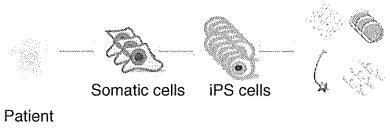

Figure 1 Generation of induced pluripotent stem cells (iPSCs) and their differentiation potential. iPSCs are derived from easily accessible somatic cells. In contrast to organ-specific stem cells, pluripotent stem cells such as embryonic stem cells and iPSCs show the ability to differentiate into many different cell types in culture. This allows *in vitro* generation of specific tissue cell types with the characteristics of the disease phenotype, from patient-derived iPSCs.

the pluripotency-defining proteins.[12] The Klf4 transgene is not necessary for reprogramming under certain conditions such as histone deacetylase inhibition[13,14] and in the absence of the tumor suppressor gene *Trp53*.[15]

The reprogramming process is highly enhanced by c-Myc,[16] although its inclusion in the reprogramming process should be discouraged, given its clear oncogenic potential. c-Myc expression is ubiquitous, in contrast to the other Myc family members, N- and L-Myc.[9] L-Myc and c-Myc mutants, all of which have little transformation activity, were shown to promote the generation of human iPSCs with more efficiency and specificity as compared with wild-type c-Myc.[7]

For these reasons, the original quartet of reprogramming factors (Oct3/4, Sox2, Klf4, and c-Myc) are not necessary under certain conditions and could be modified in accordance with the experimental context. Clearly, it is necessary to obtain a better understanding of the mechanisms underlying somatic cell reprogramming in order to fully validate the iPSC technology.

iPSC/ESC differentiation repertoire and tumorigenicity

In vitro culture and the differentiation of stem cells provide us with opportunities for disease modeling, drug discovery, and cell replacement therapy. The generation of specific functional cell types from ESCs/iPSCs has been demonstrated, including neural cells, vascular endothelia, smooth muscle cells, cardiomyocytes, hematopoietic cells, pancreatic insulin-producing cells, and hepatocyte-like cells.[17–23] The current differentiation repertoire includes more than 200 types of somatic cells.[24] These cells may be applied in regenerative medicine, and work is ongoing to overcome the remaining hurdles. Significant challenges in iPSC-based regenerative medicine include (i) the tumorigenic potential inherent to the reprogramming methods, (ii) the difficulty in achieving highly targeted differentiation, and (iii) the complexity of cellular transplantation techniques.[25]

Eradicating the tumorigenic potential of iPSC-derived cells is of fundamental importance to further enhance clinical transfer of the technology. Interestingly, the teratoma-forming propensities of secondary neurospheres, after transplantation into the brains of nonobese/severe combined immunodeficient mice, vary significantly depending on the origin of the tissue from which the iPSCs were derived.[26] For example, secondary neurospheres from iPSCs generated from adult tail-tip fibroblasts of mice showed the highest propensity for tumorigenicity, whereas those from iPSCs originating from mouse embryonic fibroblasts and gastric epithelial cells showed the lowest such propensity, the latter being comparable, in this regard, to those obtained from ESCs. Secondary neurospheres from hepatocyte iPS cells showed an intermediate teratoma-forming propensity. The use of iPSCs in regenerative medicine clearly requires further improvement of differentiation protocols in order to minimize tumorigenicity.

iPSC-BASED DISEASE MODELING

There are many potential causes for the failed translation of drug discovery from levels of molecular and animal models to human therapeutics. In particular, the success of preclinical phases of drug development is based on animal models.[27] Furthermore, <10% of the compounds that enter the clinical phase of testing reach the stage of market approval; the estimated cost of the entire drug development process is US$1.2–1.7 billion per drug.[27–29] Drug discovery/development platforms using iPSC-based disease models could be useful in filling the gap between animal models and clinical trials.

iPSC technology is expected to provide innovative tools for drug development via high-throughput therapeutic/toxicity screening, using differentiated cells from patient-derived iPSCs. This disease-modeling approach to drug discovery will also increase our understanding of disease progression and biology in specific cell types, which could possibly lead to redefining known aspects of diseases.[30] Patient-specific iPSCs provide not only genetic information but also potential phenotype attributes. In addition, iPSCs can be generated from patients irrespective of whether the disease is in the familial or the sporadic form. Drug screening platforms can be developed to test compounds (including biologics such as small hairpin RNAs) that are able to make the disease-related phenotype revert to that of the non-disease control.[30]

The available lines of human ESCs are variable with regard to epigenetic information, expression profile, and differentiation propensity.[31,32] Significant intrinsic variability also remains in iPSC lines, and abnormal expression of imprinted genes has been detected in a significant number of them.[33] These inter-iPSC differences were attributed to the introduction of reprogramming factors using randomly integrating viral vectors, and/or to persistent donor cell gene expression.[34] However, even if iPSCs are generated in the absence of integrating factors, intrinsic variability remains,[35–37] including in the matter of neuronal differentiation competence.[38] Moreover, expression profile analysis of integration-free human iPSCs has shown an expression signature in iPSCs that is distinct from those of both the original population and standard human ESCs.[35] It is also reported that there is a strong correlation between gene expression signatures and specific laboratories, in both ESC and iPSC lines, because of differences in the *in vitro* microenvironment.[39] These observations suggest that further dissecting the intrinsic variability of iPSCs may provide clues regarding the wild-type iPSCs that would be most suitable as experimental controls and the number of control lines that should be obtained for each experiment.[35] Despite these variations, however, many

Table 1 Disease modeling using disease-specific iPSCs

Disease (responsible gene)	Inheritance pattern	Age of onset	Recapitulated phenotype/proof of drug efficacy
Spinal muscular atrophy[41] (*SMN1, SMN2*)	AR	Infancy to adolescence	1. Decreased no. (%) of ChAT$^+$/Tuj1$^+$ neurons 2. Decreased SMN protein level (evaluated with WB/IA) 3. Rescue phenotype 2 with 1 mmol/l valproic acid
Familial dysautonomia[42] (*IKBKAP*)	AR	Infancy	1. Increased abnormal splicing in differentiated neural crest 2. Decreased no. (%) of ASCL1+, Tuj1+ neurons 3. Migratory dysfunction (scratch assay) 4. Partial rescue phenotype 1, 2 with 100 μmol/l kinetin
Fanconi anemia[43] (*FANC A~N*)	AR/XR	First decade ~4/5th decade	1. Unsuccessful at obtaining iPSCs from patient's fibroblast → after "*in vitro* genetic correction" of patient's fibroblast Successful in obtaining iPSCs (chromosomal instability) 2. Differentiate into CD34+/hematopoietic progenitors
Dyskeratosis congenita[44] (*XR: DKC1*)	XR (AR/AD)	Adolescence	1. Elongated telomere in iPSCs (TERT/TERC↑) 2. Shortened telomere after differentiation (TERT/TERC↓)
LEOPARD syndrome[45] (*PTPN11, RAF1, SHOC2*)	AD	Infancy to adolescence	1. Enlarged cell size of differentiated cardiomyocyte 2. Inactivated RAS-MAPK pathway (bFGF induction)
Rett syndrome[46] (*MeCP2*)	XR	6–18 Months	1. Reduced no. of glutamatergic synapses and morphological alterations (synapsin puncta at dendrites), rescued by IGF-1 (ng/ml) 2. Reduced RTT protein level/cell size and rescue by gentamicin (100 μg/ml) at Q244X clone 3. Reduced activity-dependent calcium transients 4. Reduced spontaneous postsynaptic currents

AD, autosomal dominant; AR, autosomal recessive; IA, immunological analysis; IGF-1, insulin-like growth factor 1; iPSC, induced pluripotent stem cell; TERC, telomerase RNA component; TERT, telomerase reverse transcriptase gene; WB, western blot analysis; XR, X-linked recessive.

lines of disease-specific iPSCs are being generated,[40] given that several studies have actually recapitulated the phenotypes of diseases in the iPSC-derived targeted cell population and that this approach now finds a place on the drug development platform as a useful tool to complement *in vivo* experiments (Table 1).[41–46]

To avoid both inter- and intrapatient clonal variations of iPSCs, it is necessary to purify targeted cells by fluorescence-activated cell sorting or magnetic sorting using fluorescent or magnet-labeled antibodies[27] or by high-content analysis.[47,48] The control of the prominent heterogeneity of iPS-derived differentiated cells presents a technological challenge; this continues to be the major limitation of standardized high-throughput screening, although further modifications in differentiation protocols are under way in our laboratory.

iPSC-BASED TOXICITY SCREENING

The progressive attrition of medicinal products in the long pipeline between "hit" identification and the market has become one of the concerns of the pharmaceutical industry in the past decade.[48] The development cost of a marketable product is continuing to grow.[27–29,49] In 2001, development was abandoned because of lack of efficacy in 30% of the medicines that entered clinical trials and in another 30% because of safety concerns[49] such as cardiotoxicity and hepatotoxicity. The effective development of new drugs therefore requires predictive toxicity assays of adequate accuracy during preclinical testing. The use of human iPSCs and robust protocols to differentiate them into cardiomyocytes and hepatocytes should be able to provide straightforward assays for analyzing certain aspects of drug metabolism and for assessing probable side effects. However, technological hurdles still exist with respect to achieving the desired maturity of differentiated cells[50] and minimizing the substantial heterogeneity of iPS-derived differentiated cells for the assay. Despite these limitations, significant progress has been made.

The drug-induced blockade of the ether-a-go-go related gene 1 (*hERG1*) channel is reportedly associated with an increased duration of ventricular repolarization, causing prolongation of the QT interval (i.e., long-QT syndrome).[51–54] Data related to the electrophysiological capacity and responsiveness of human iPSC-derived cardiomyocytes in response to several cardiac and noncardiac drugs have been reported.[51–54] Cardiac toxicity screening tools based on these approaches will soon become available.

The efficient generation of functional hepatocyte-like cells from iPSCs has been also reported.[20,21] The use of three-dimensional culture as well as co-culture systems (e.g., associating Kupffer and/or endothelial cells with hepatocytes in order to mimic the *in vivo* hepatic context) are among the strategies now recognized to enhance the generation of even more mature cells.[49]

To establish toxicity screening tools using iPSC technology, validation is essential. In particular, it is crucial to show high fidelity of the iPSC-based toxicity screening tools in reproducing, *in vitro,* the toxicity profiles of "hit" drugs that had been eliminated from the development pipeline because of safety concerns.

CHALLENGES IN iPSC-BASED APPROACHES

Aging process and environmental effects

Several diseases that are characterized by onset in early life have been successfully modeled using iPSC technology.[41–46] On the other hand, in some diseases (including neurodegenerative

diseases) that are age dependent, patient-specific iPSC-derived neural cells may not immediately manifest the disease phenotype as compared with normal control cells, under basal cell culture conditions.[30,55,56] This may also apply to drug toxicity that shows age-dependent susceptibility. Identification of disease/toxicity-related phenotypes in short-term settings *in vitro* appears particularly challenging, but it may be possible to achieve by mimicking the aging process with stressors such as oxygen reactive species, proinflammatory factors, or toxins.[30,55,56] Identification of new and more effective and relevant stressors that can accelerate the process of eliciting phenotypes in models of late-onset diseases will therefore be an important goal for future disease modeling.[30,55,56]

Even patients with monogenetic diseases manifest large genotype–phenotype variability. Therefore, it would be more difficult to establish disease modeling from sporadic-disease iPSCs, given the complexity of the different genetic backgrounds and environmental cues involved.[27,30] It will be both challenging and exciting to examine whether the same phenotype as seen in monogenic-disease modeling could be recapitulated in sporadic-disease-iPSC-derived modeling by reproducing environmental effects *in vitro*.[27,30,55,56]

Definition of "control"

Whether in selecting a therapeutic or in toxicity assays using patient-specific iPSC-derived cells, the use of well-defined, non-disease control cells is crucial. Recent genome-wide association studies[57] have demonstrated that every person has disease-relevant single-nucleotide polymorphisms, and it is therefore impossible to categorically define iPSCs that represent perfect non-disease control.

Nonetheless, we think that the following two approaches are valid for deriving iPSC-positive (disease) and negative (non-disease) controls: (i) deductive and (ii) inductive. Deductive controls would include non-disease iPSC/ESC lines with modification (e.g., disease gene transgenic and disease gene knock-in), disease gene–corrected iPSC/ESC lines generated from disease iPSC/ESCs, and iPSCs with non-disease alleles from an individual patient in somatic mosaicism (Table 2). Deductive approaches define negative and positive controls in similar genetic backgrounds, providing benchmarks of disease modeling to specify differences between disease and non-disease control, whereas contributors other than the targeted gene(s) are not considered. On the other hand, inductive controls may be non-disease iPS cell lines or iPSCs from healthy individuals or from other patients (positive control). This approach could be less complicated than the deductive method, especially if noise from iPSC variations can be further reduced.

For the deductive control setting of disease modeling, the tools for achieving expression or knockout of disease genes in hiPSCs/ESCs by random integration of vectors (including viruses, bacterial artificial chromosomes, synthetic gene delivery reagents, and a transposon/transposase system) are useful.[58–60] Also, the current development of engineered nucleases makes targeted genome modification an attractive tool with therapeutic potential that may go beyond the development of drug screening tools.[58]

Table 2 Proposed definition of "control" in induced pluripotent stem cell research

Deductive approach
Embryonic stem cell line with and without disease-introducing genetic modification
Non-disease induced pluripotent stem (iPS) cell line with and without disease-introducing genetic modification
Disease iPS cell with and without disease-correcting genetic modification
iPS cell from somatic mosaic with and without disease allele
Inductive approach
iPS cell from a patient and a disease-free family member
Disease genetic risk-ascertained iPS cell lines (preferably as a risk-absent non-disease control)
iPS cell lines from disease-phenotyped individuals (healthy or disease control)

iPSC-BASED NOVEL DRUG DEVELOPMENT PLATFORM

iPSC-based *in vitro* phase III

Diseases can be divided into rare, monocausal genetic diseases and a large group of sporadic, multifactorial diseases. No large-scale disease modeling is currently available for the latter group. Technological advances in rapid and easy iPSC generation on a large scale will realize the possibility of both *in vitro* phase III and case–control studies by using non-disease and disease controls derived from age/gender-matched donors or from family members regardless of age/gender.[30] One of the factors facilitating the process could be to obtain a blood sample from each patient in order to generate iPSCs. iPSC generation from peripheral blood drops from each patient would allow case–control studies to be carried out, although several issues must still be resolved prior to the use of iPSCs from peripheral blood cells.[61–64] First, the differentiation potency of these iPSCs must be analyzed further.[61] Peripheral blood–derived iPSCs may preserve epigenetic memories of having been blood cells and may therefore exhibit preferred differentiation into hematopoietic lineages rather than into other cell types.[61,65]

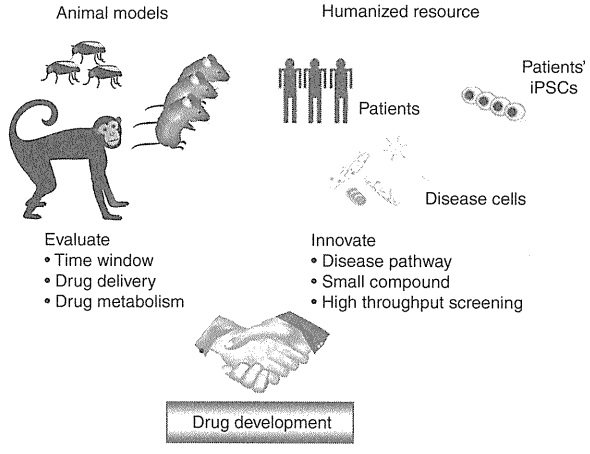

Figure 2 Combined approach involving animal models and induced pluripotent stem cell (iPSC) technology. The new iPSC technology is complemented by a drug development strategy in preclinical settings that uses animal models and other conventional approaches.

Another issue is how long and to what extent iPSC clones from terminally differentiated cells can be expanded.[61–64] Finally, the effect of the presence of pre-existing T-cell receptor rearrangements on the properties of iPSC or differentiated cells needs to be determined.[61,66] Besides minimizing the invasive biopsy procedures, reducing the time required for iPSC differentiation, resulting in lower costs, would be essential for large cohort studies, potentially leading to the discovery of novel drug targets.

iPSCs and animal models

Cell lines and animal models contribute to the exploration of disease mechanisms and drug development for various diseases. However, the animal models do not always demonstrate the same phenotypes as those seen in humans.[55] For instance, in mice the type and/or distribution of cardiac ion channels are different from those in humans, demonstrating a relatively shorter duration of action potential and higher heart rate (600 bpm).[67] An *in vitro* analysis of human cardiomyocytes is therefore critical to an understanding of the mechanism of genetics-related arrhythmias in humans.[67] Also, compounds that demonstrate significant benefit in animal models may fail to show effectiveness in clinical trials in humans.[55,68,69] The use of transgenic mice of mutant superoxide dismutase (*SOD1*), a gene found to be associated with amyotrophic lateral sclerosis,[70] enabled the identification of several compounds that relieve the disease phenotype, including vitamin E and creatine.[71–73] However, when these compounds were tested in humans, no clinical improvements were observed.[71–73] The toxicity of compounds is sometimes missed in cell lines and animal models because specific interactions with human biological processes cannot be recapitulated in these systems.[27] Also, the use of animal models for toxicity assays may be ethically problematic, the animals may be expensive to purchase and maintain, and the process may be difficult to automate.[27] Clearly, we require different drug screening models that complement these systems and represent the human condition with high fidelity.[74] iPSCs are expected to fulfill these requirement and are amenable to the demands of drug development. There are nonetheless great advantages associated with cell line–based models (which could be used for homologous culture, yielding reproducible results) and for animal models (which provide information regarding optimal time window, drug delivery, metabolism, etc.) (**Figure 2**). Integrated drug screening systems, consisting of disease-specific iPSC-based models as well as cell lines and animal models, would greatly enhance the efficiency of translational drug research.

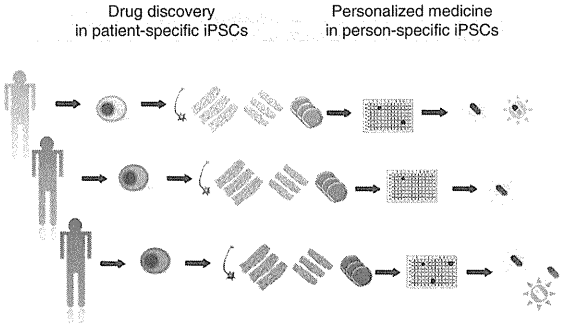

Figure 3 Personalized medicine based on induced pluripotent stem cell (iPSC) technology. iPSC technology is highly amenable to individualized approaches. Person-specific iPSCs can be derived, differentiated into specific cell types, and used for therapeutic/toxicity response assays.

Personalized medicine

The striking advantage of using iPSCs rather than ESC-based approaches is that iPSCs can be derived from any individual with relative ease, thereby allowing development of a personalized study platform on individual genomic information. iPSCs and

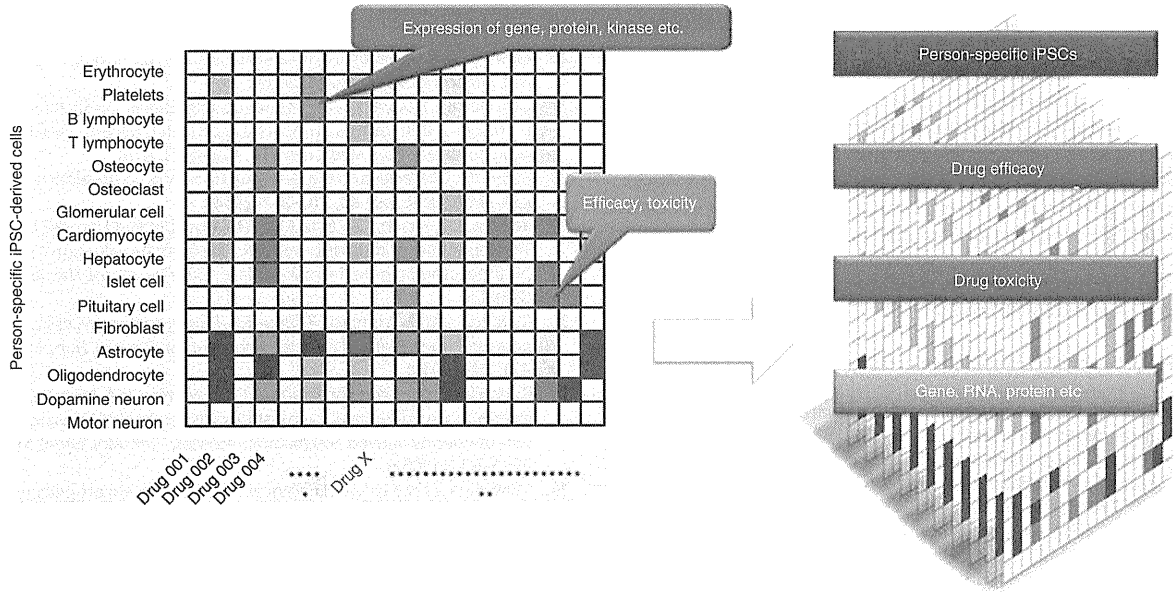

Figure 4 "Pharmaco-iPSCellomics" by person-specific iPSCs. iPSCs derived from individual subjects/patients can be differentiated into multiple cell types, thereby providing a personalized iPS-cellome platform. This cell-based system can be used for drug discovery and selection of clinical therapeutics with various biomarker end points.

differentiated cells from the iPSCs retain their personal identity, like an alter ego, suggesting that iPSC technology can be applied to disease-, patient-, and finally person-specific approaches to examine the individual differences in pharmacokinetic/pharmacodynamic features (Figure 3). Given that everyone will almost certainly become a patient at least once in his or her lifetime, individual iPSC-based predictive therapeutic and toxicity profiling of all drugs available in multiple cell types will be a logical and attractive approach. This "pharmaco-iPSCellomic" analysis (Figure 4) could eventually be available in an array-based format for high-throughput assay before specific drug therapy is prescribed for a particular disease condition.

CONCLUSION

The potential of iPS cell technology in drug discovery is enormous.[75] At the same time, the technology is still in its infancy with numerous challenges to overcome before its clinical translation is complete. The long journey has just begun. It may take years to reach the eventual goals, but the iPSC technology itself, combined with existing methods and models, will begin to contribute to the development of new cures.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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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 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

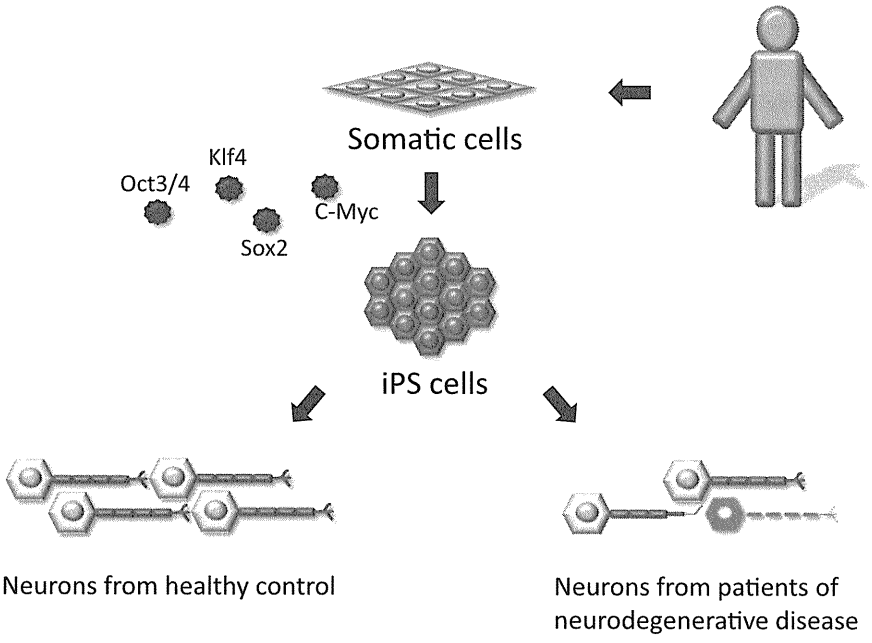

Figure 1 Modelling of neurodegenerative diseases using patient-specific induced pluripotent stem (iPS) cells. c-Myc, myelocytomatosis oncogene; Klf4, Krüpel-like factor 4; Oct3/4, octamer-binding transcription factor 3/4; Sox2, sex determining region Y-box 2.

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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82

iPS細胞作製技術を用いたALS治療法開発

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1．はじめに

筋萎縮性側策硬化症（ALS）におきまして、病変の首座は、運動ニューロンといわれる、体の随意運動をコントロールする神経細胞にあります。運動ニューロンは、頭蓋骨や脊椎骨で囲まれた中枢神経系に位置するため、直接に病態を解析するには限界がありました。そのため、遺伝学的解析、病理組織・遺伝子改変動物/細胞モデルなどの生化学的・組織学的解析を中心にこれまで研究が進められてきました。すなわち、患者さんの病態を間接的にしか解析することが出来ませんでした。

2006年に人工多能性幹細胞（induced Pluripotent Stem cells: iPS細胞）の作製技術が開発されました。そのことにより患者さんご自身の皮膚の細胞から、患者さんご自身の遺伝情報を有する運動ニューロンを含めた中枢神経系の細胞を入手することが可能になりました。そのことによって、これまで知られていなかったALSという疾患の新たな病因が明らかになる可能性が生まれました。また、患者さんご自身の細胞を生きたまま入手することができることから、培養皿の中でALSという疾患の病態が再現できる可能性が生まれ、病態解明・創薬開発がすすむことが期待されています（図）。

本稿では、現在までのiPS細胞研究の基礎的側面、疾患研究への応用、iPS/ES細胞を用いたALS研究、今後の展望について述べさせていただきます。

2．iPS細胞研究の基礎的側面

様々な細胞に分化できる細胞を、多能性幹細胞といいます。多能性幹細胞には、胚性幹細胞（Embryonic Stem cells: ES細胞）やiPS細胞などがあります。

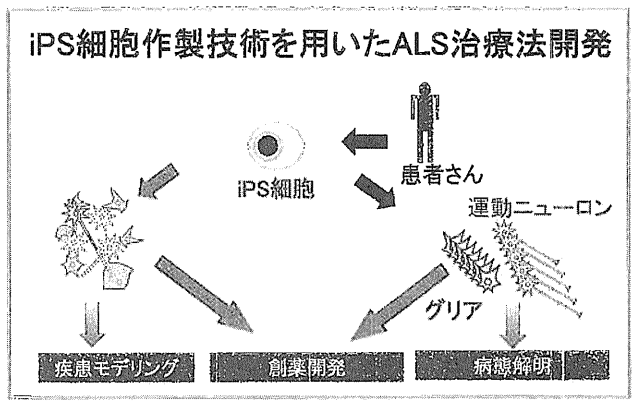
ヒトES細胞は、1998年に初めて樹立されました。しかし、ヒトES細胞は、受精後の発生初期の胚の一部を用いて樹立されるため、多くの疾患から樹立することは、現時点では困難です。そしてES細胞に特徴的に発現しているOct3/4・Sox2・Klf4・c-Mycという遺伝子の皮膚線維芽細胞への導入により、2006年にマウスで、2007年にはヒトでES細胞類似の多能性幹細胞樹立がなされ、iPS細胞と命名されました。

現在では、皮膚以外に、血液、胃等、体内の様々な部位から、また、ウイルスを用いる方法以外に、DNA、RNA、小分子化合物を用いる方法等、様々な方法での作製が報告されています。今後は、侵襲ができるだけ少なく、効率よく、作製にかかる期間が短い方法での樹立方法開発が進むと考えられます。

3．疾患研究への応用

iPS細胞誕生以降、いくつかの疾患で、患者さん由来のiPS細胞を用いた疾患モデリングが報告されています。疾患モデリングとは、iPS細胞から分化誘導した細胞が、培養皿の中で実際の病気と同様の症状になることです。

これまで、患者さんのiPS細胞を用いて、疾患モデリングに成功した神経疾患には、脊髄性筋萎縮症、家族性自律神経失調症、レット症候群等があります。それぞれ疾患に罹患する神経細胞の細胞数減少等、疾患表現型などを培養皿の中で再現し、さらには既存薬剤による疾患表現型の改善を示すこと等に成功しています。このことは、その培養皿の中のモデルが、新たな薬剤をスクリーニング（選別していくこと）することに用いることができる可能性を示して



います。

4. iPS/ES細胞を用いたALS研究

1993年、家族性ALSの一部の家系で、SOD1という遺伝子に変異があることが明らかになりました。1994年、ヒトの変異SOD1遺伝子を有するマウス（変異SOD1マウス）が作製され、ヒトALSと同様の症状を呈することが明らかになりました。その際に、治療法開発が一気に加速することが期待されましたが、実際にはマウスで有効だったほとんどの薬剤が、患者さんでは有効ではありませんでした。その一つの原因としては、ヒトとマウスの細胞が、異なっていること（細胞内部の信号伝達等の違いによる可能性等があります）が考えられました。よって、ヒト、しかも患者さんご自身の細胞を用いた創薬開発が求められていました。そのような状況の中、ヒトiPS細胞が誕生しました。

しかしながら、変異SOD1マウスを用いた研究により、多くのALSの病因も明らかになっています。その中でも重要な病因として、脊髄運動ニューロン自体だけではなく、その周辺の変異SOD1を有するグリア細胞が、脊髄運動ニューロン細胞死を増強、加速していることが明らかになりました。2007年ハーバード大学医学部のケビン・エッガン博士らのグループや、コロンビア大学のグループが、変異SOD1マウスから作製したグリア細胞が、マウス脊髄運動ニューロンの細胞死を生じることを報告しました。2008年には、エッガン博士のグループや他のグループが、ヒトES細胞から分化誘導した脊髄運動ニューロンを変異SOD1を有するグリア細胞と一緒に培養すると、それが正常のヒトES細胞由来脊髄運動ニューロンであっても、細胞死に至ることを報告しました。そのことは、変異SOD1を有するグリア細胞が脊髄運動ニューロンに対する毒性を有していることを示しています。その報告の中で、プロスタグランジンといわれる生理活性脂質がグリアの毒性に関連しており、創薬の標的となりうることが示されています。本年度、国際幹細胞学会において、エッガン博士は、ヒトES細胞から分化誘導した脊髄運動ニューロンをGFPというクラゲ由来の蛍光タンパク質で光らせ、培養皿の中で変異SOD1を有するグリア細胞と一緒に培養し、タイムラプスイメージングという動画撮影で運動ニューロンの細胞死が起こる瞬間の映像を、世界で初めて報告しました（日本のTV番組でも放映されました）。

2008年、エッガン博士らのグループは、高齢のALS患者さんの皮膚線維芽細胞からiPS細胞を作製し、さらにiPS細胞から脊髄運動ニューロンまで分化誘導が可能であることを報告しました。しかし、その後、世界において、未だ、ALS患者さんのiPS細胞を用いた疾患モデリングに関する報告はありません。iPS細胞を用いて疾患モデリングに成功した先述の疾患とALSとの違いには、①ALSが比較的高齢になってから発症する疾患であること（加齢変化）、②孤発性ALSの場合には遺伝的背景以外の環境の影響が発病に関連している可能性があること（環境要因）、③ALSではグリア細胞等の脊髄運動ニューロン以外の細胞が脊髄運動ニューロン細胞死への強い影響を有する可能性があること（周辺細胞の毒性）、の3点が挙げられます。私達の研究室も含め、これらの三つの要素を巧みに取り入れた培養皿の中での疾患モデリングの試みを、世界中の研究室が行っています。

これまで、私達の研究室では、疾患モデリングの試みに加えて、多くの大学、製薬会社等と共同で、脊髄運動ニューロンやグリア細胞と他の細胞ができるだけ混じらないように純化し、大量に入手する方法を開発しています。現在、患者さん由来の脊髄運動ニューロンやグリア細胞の詳細な解析を行い、新たな創薬の標的を同定しつつあります。その中でも、変異SOD1によるALSに対しては複数の治療薬候補を選定すると同時に、動物モデルを用いた検証に

も取り組み始めています。

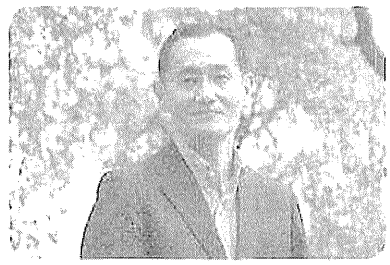
5. おわりに

iPS細胞作製技術というこれまでの歴史上に存在しなかった革命的技術を用いることによって、培養皿の中でのALSの疾患モデリング、病態解明、創薬開発が加速しています。あらゆる可能性、考えられうるすべての手法、分野を超えて協力しうるすべての研究者との共同研究によって、世界中の研究者も私達も、全速力で、ALSという疾患の制圧を目指しています。

最後になりましたが、本研究にご協力いただいております患者さん、ご家族の皆様、そして協会の方々に、心より御礼申し上げます。どうぞ今後とも何卒よろしくお願い申し上げます。

iPS細胞研究所　山中伸弥所長より JALSA会員への メッセージをいただきました。

京都大学iPS細胞研究所（CiRA）では、井上治久准教授を中心にALSの患者さんから提供していただいた細胞からiPS細胞を作製し、それをさらに分化させた運動神経細胞等を用いて、治療薬探索の準備を進めています。他の研究者や製薬企業とも協力しながら研究を強力に推進し、新しい治療法の開発に貢献したいと思います。

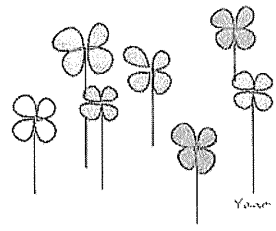


トピックス

メコバラミンの治験　中間解析報告

ALS治療薬として治験を行っているメコバラミンについて、エーザイ株式会社より以下の中間解析報告を受領しましたので紹介致します。

メコバラミンのALSに関する治験は，2006年より開始し、既に患者様の登録は2010年8月末に締め切らせて頂いております。その後、2010年10月末に本剤の有効性及び安全性に関する中間解析が、エーザイとは独立した効果安全性評価委員会によって実施されました。その結果に基づき、同委員会において当該治験の継続可否について検討が行なわれ、有効性及び安全性に関する中止基準に該当しなかったため、弊社は同委員会より「継続」の旨の勧告書を受領いたしました。当該勧告書に基づき、本治験を引き続き継続させて頂くことを決定致しましたので、ご報告申し上げます。なお、当該中間解析は弊社とは完全に独立した形で実施されておりますため、弊社は解析結果の詳細について一切知らされておりません。この点につきましてご了承下さいますよう何卒宜しくお願い申し上げます。



第1回

ALSフォーラム

～ALS最前線～

記録集

2010年8月28日
東京ドームホテル

サノフィ・アベンティス株式会社

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Because health matters

2010年12月作成

❶-4

iPS細胞作製技術を用いた神経変性疾患の研究

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井上　治久

ヒトiPS細胞は2007年に誕生した。ヒトの皮膚細胞にES細胞（胚性幹細胞）に特徴的な転写因子を3～4個導入することによって、ヒトの皮膚細胞がES細胞様の細胞に初期化されることが発見された。

この技術を応用して神経変性疾患においては、患者の細胞からiPS細胞を樹立し、神経細胞やグリア細胞に分化誘導することによって、創薬もしくは移植医療への応用、あるいは病態を再現することができれば、病態解明につながることが期待されている。今回は、iPS細胞を用いた神経変性疾患に関して、現在我々が進めている研究も含めて紹介する。

1. 疾患の再現、薬物スクリーニング

ハーバード大学の研究者らは2008年に、SOD1変異のある高齢ALS患者から採取した皮膚線維芽細胞からiPS細胞を樹立し、さらに運動神経細胞への分化誘導に成功した[1]。また同年、ウィスコンシン大学のグループはSMN1遺伝子の変異によりスプライシングの異常をきたし、運動神経が特異的に減少する脊髄性筋萎縮症患者の線維芽細胞からiPS細胞を作製し、分化誘導した運動神経において神経の減少を再現し、有効性が証明されている薬剤を作用させると、蛋白質SMN1量を増加することを報告した[2]。また、1-κ-B kinase complex associated protein（1κB AP）遺伝子変異によって発症する家族性自律神経失調症から作製された自律神経の前駆細胞（神経提細胞）では、遊走能異常や異常遺伝子発現が再現されたほか、既知の薬剤の有効性が確認された[3]。

そこで我々は、SOD1変異ALS患者の病態解明のためのiPS細胞を樹立する前に、ヒトiPS細胞を運動ニューロンに分化誘導しうるかを確認した。SOD1変異ALS患者の細胞からiPS細胞クローンを10～30個樹立し、ES細胞のマーカーを発現していること、さらに導入遺伝子が10%以下になるようにサイレンシングされていることを確認し、確認できたクローンについて核型を検討した。同時にALS患者が有していたSOD1遺伝子変異が引き継がれていることを確認した後、免疫不全マウスの中で3胚葉に分化するES細胞の表現型を有していること、運動神経にも確実に分化誘導できることを確認してから実験に用いた。

2. Cloning variationとcloning selection

iPS細胞は分化誘導効率や、増殖速度が異なることから、clonal varietyがあるといわれる。このclonal varietyの原因としては、様々な要因が考えられている。我々は、導入遺伝子が確実にキャンセルされているか（サイレンシング）を指標にアンバイアスでクローンを選択した。

3. 神経細胞死を標的とした薬物スクリーニング

変異SOD1を有する星状膠細胞が毒性を有していることはヒトES細胞から分化誘導された運動ニューロンですでに証明されている。我々は、変異SOD1を有する星状膠細胞上で、SOD1変異ALS患者の運動神経、もしくは変異を有さない運動神経を標識し共培養を行った。確実に神経変性が生じるかをできるだけバイアスのない状況下で、1つひとつをできるだけ高速で、どれくらいの細胞が減少しているのかを現在解析している。

また、前述の非自律性神経変性で問題となる星状膠細胞の毒性については、SOD1変異ALS患者、変異を持たない患者（対照）数名ずつから星状膠細胞を誘導し、同定法の1つであるマイクロアレイを用いて遺伝子変異の違いがある遺伝子を同定した。

4. 封入体を標的とした薬物スクリーニング

一方、SOD1と同様、重要な遺伝子であるTDP-43遺伝子変異については、TDP-43変異ALS患者から誘導したヒトの運動神経の解析を行っている。

ヒトのリソースとしてiPS細胞を利用することにより、今後はさらに新しい情報が見つかることが考えられる。

参考文献

1）Dimos JT, et al. Science 321：1218-1221, 2008
2）Ebert AD, et al. Nature 457：277-280, 2009
3）Lee G, et al. Nature 461：402-406, 2009

Session ❶ 討論

1) TDP-43

座長 ヒトTDP-43を発現するトランスジェニックマウスモデルの表現型に関連して、マウス脳組織にも細胞培養と同じような変化がありましたか？

野中 運動機能異常のような表現型が出ていたので、6ヵ月ぐらいのときに一度、脳を観察したのです。患者脳でみられるようなリン酸化TDP-43抗体に陽性な凝集体は脳でも脊髄でもほとんど存在していませんでした。海外のいくつかのグループも同様の結果でした。今後は、マイクロアレイなどで解析してみてはどうかと考えているところです。

会場 蛋白質というのは凝集を形成すると思うのですが、それらが野生型のTDP-43を巻き込むようなことが起こり得るのかどうかについてご教示いただけますか。

野中 TDP-43以外の、タウ、シヌクレインの凝集体形成モデルも研究しているのですが、TDP-43は断片化すれば、発現しただけで凝集体ができますが、タウやシヌクレインは発現させただけでは凝集体はできません。シヌクレインを発現したところにタウの線維を入れても凝集しないことから、シヌクレイン同士、タウ同士という組み合わせがあるようで、TDP-43に関しても同様にクロスシードしなかったというデータが出ています。

2) FUS/TLS

会場 塩基性封入体は、従来いわれている塩基性封入体とはちょっと違うように思いますが、ユビキチンがよく染まるといった特性はありますか。

会場 ユビキチンに関しては変化しやすく、遺伝性のものがよく染まるように思いますが、それ以外ではなかなか染色の差が出ていません。また塩基性封入体を染色する際、遺伝性がないほうが染色されにくいという感触はあるものの、形態は多彩だと思われます。

青木 おそらく、FUS/TLS遺伝子異常がある家系での塩基性封入体とない家系での封入体が見分けられるようになれば、染色の差も明らかにできるとは思いますが、現時点ではまだはっきりしていません。

3) Optineurin

会場 Optineurinは緑内障も遺伝性に起こすファクターとのことですが、緑内障を起こすメカニズムとALSを起こすメカニズムとの間に、何か共通したファクターがあるのでしょうか。臨床医としては、神経系にoptineurinが沈着するようであれば目の症状に注意する必要があるのでしょうか。

川上 緑内障に関しては、最初は分泌された異常蛋白が前房で詰まって眼圧が上がるという説が唱えられていました。しかし、前房にはoptineurinが分泌されないことが明らかになり、最近、岐阜大学でトランスジェニックマウスが作成されました。メカニズムの説明に関しては、Rab8との相互作用が悪くなって、運送されなくなって緑内障が発症するといわれています。ALSにおける変異に関しては、Rab8の相互作用はみていませんが、NF-κBに対する作用が異なるので、機序的には違うものと考えています。

会場 Optineurinで染めてみると、SOD1や孤発性ALSも染まりますし、非常に広い範囲のものが染色されるということで、共通の経路にoptineurinがあるとのことですが、経路のかなり下流でoptineurinが関係しているというエビデンスはあるのでしょうか。

川上 推測ですが、まず1つはSOD1の場合、主な原因はSOD1変異であることが明らかなため、共通なメカニズムではあるものの、より末梢のところでエフェクターとなっている可能性が考えられます。もう1つは、細胞内の物質輸送に絡んでいるため、封入体処理や封入体形成のときに関係している可能性はあると思われます。

4) iPS細胞

会場 ALSなど成人発症型の疾患の場合に、iPS細胞を作製して神経分化をさせた時点で、すでに表現型の違いはあるのでしょうか。もしないとすれば、その点をどう解決すれば疾患モデルが作製できるか教えてください。

井上 重要なポイントです。これまで疾患モデル開発に成功している疾患ではほとんどが劣性遺伝形式で、