況および今後の課題について述べる（**図 1**）.

## I. *in vitro* での iPS 細胞の活用

### I. 疾患モデリング

iPS 細胞技術が開発されて以来，さまざまな疾患特異的 iPS 細胞を用いた *in vitro* での疾患モデリング（疾患再現）が報告されている．それらの中で神経・精神疾患に関する報告を**表 1** にまとめた.

早発性疾患は原因遺伝子の影響が大きいと考えられ，脊髄性筋萎縮症（spinal muscular atrophy: SMA）[5]・家族性自律神経失調症（familial dysautonomia: FD）[6] などでいち早く疾患再現が報告された．SMA では疾患 iPS 細胞由来運動ニューロンの減少や患者由来線維芽細胞・iPS 細胞での核ジェム数の減少が示された．また，FD では疾患特異的 iPS 細胞由来神経堤細胞の移動度の減少が示された.

その後，晩発性疾患であるパーキンソン病[7] で，疾患特異的 iPS 細胞由来ドーパミン作動性神経細胞を酸化ストレスの一種である過酸化水素や 6-OHDA に曝露すると，コントロール細胞と比較し神経細胞死が誘導されやすい，つまり酸化ストレスに対し脆弱であることが示された.

また，精神疾患である統合失調症[8] の疾患再現が報告された．細胞レベルでの表現型が不明な疾患ではあるが，コントロール iPS 細胞および疾患特異的 iPS 細胞からシナプシン陽性神経細胞を分化誘導し，疾患特異的 iPS 細胞から分化誘導した神経細胞のシナプス結合が減少していることを示した．さらに，抗精神病薬の一つであるロキサピンを培養液に添加することにより，減少していたシナプス結合がコントロール細胞と同程度にまで増加した．このように，多因子で生じる疾患や細胞レベルでの表現型が不明な疾患であっても，疾患 iPS 細胞から分化誘導した神経細胞をコントロールと比較することでモデルを構築することが可能

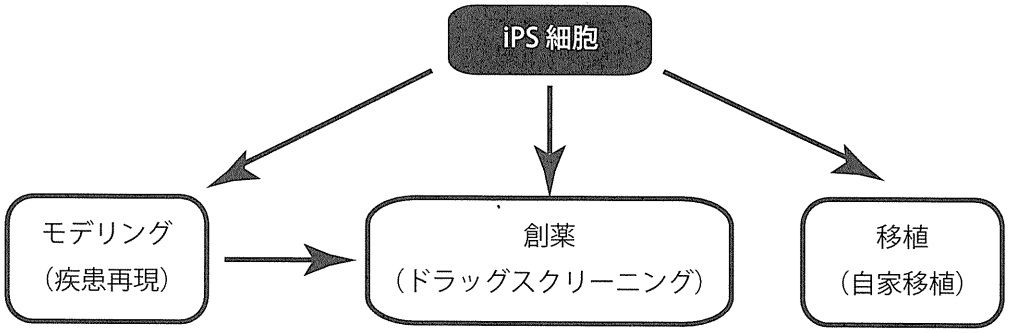


**図 1　iPS 細胞の活用法**

**表 1　神経疾患特異的 iPS 細胞を用いた疾患モデリング**

| | | 報告年 | 疾患名 | 標的細胞 | 疾患モデル |
|---|---|---|---|---|---|
| 神経変性疾患 | 早発性疾患 | 2009 | 家族性自律神経失調症 | 感覚神経・自律神経 | 遊走異常 |
| | | 2009 | 脊髄性筋萎縮症 | 下位運動ニューロン | 運動ニューロン死 |
| | 晩発性疾患 | 2011 | パーキンソン病 | 中脳ドーパミン神経細胞 | 酸化ストレスに対する脆弱性 |
| 精神疾患 | | 2010 | レット症候群[9] | 不明 | シナプスの減少 |
| | | 2011 | 統合失調症 | 不明 | シナプス結合の減少 |

であることが示された点で iPS 細胞の有用性が大きく示された.

## II. 創　薬

疾患モデリングが成功した神経疾患の中で，薬剤スクリーニングが進んでいる疾患が先述した SMA である．SMA は SMN1（survival motor neuron 1）遺伝子の変異が原因で，重症度の高い患者ほど SMN たんぱく質の産生量が著しく低下している．SMN2 遺伝子は SMN1 遺伝子と非常に相同性が高いものの，一部の塩基配列の置換が原因で，SMN2 遺伝子から産生されるたんぱく質の約 90％はエキソン 7 が欠失した SMN たんぱく質である．エキソン 7 のスキッピングを修正する薬物は SMA 患者での全長 SMN たんぱく質の産生量を増加させ，SMA の治療薬になり得る可能性がある.

実際に，SMA で疾患再現・核ジェム数の減少を報告した論文では，ヒストン脱アセチル化酵素阻害作用を有するバルプロ酸およびトブラマイシンが疾患 iPS 細胞での全長 SMN たんぱく質量を増加させることを示している.

## III. 今後の課題

創薬開発を目的とした薬剤スクリーニングを行うためには，同時に多数のサンプルを高速で解析する必要がある．また，疾患の標的細胞への高効率な分化誘導法を確立し，各疾患のモデルを構築する必要がある．そのことによって，今後，他の疾患においても，薬剤スクリーニングのためのプラットフォームの創出が可能になるであろう.

さらに，晩発性疾患や精神疾患の大部分は原因遺伝子が不明の孤発性が大部分を占める．家族性疾患から得られた知見を孤発性疾患に応用することが iPS 細胞作製技術を最大限に活用することになると考えられる．そのためには，均一な iPS 細胞株を使用する必要がある．現在ではホストのゲノムへの遺伝子挿入がない方法での iPS 細胞の樹立が行われている．しかしながら，iPS 細胞は ES 細胞と類似の性質を有しているものの，DNA のメチル化の状態は ES 細胞と異なることが示されている[10]．また，iPS 細胞は体細胞コード領域の変異の起こる頻度が高いこと[11]，オリジナルの細胞とは異なったコピー数の多型が検出されること[12]が報告された．これらが，iPS 細胞のクローン間や iPS 細胞株間の多様性の原因であると考えられる（**表 2**）．今後，これらの課題を克服する技術が開発されるであろう.

**表 2　iPS 細胞技術の課題**

| | 体細胞コード領域変異 | コピー数多型<br>(copy number variation: CNV) | エピジェネティック修飾 |
|---|---|---|---|
| 頻度 | 線維芽細胞と比較し，iPS 細胞での変異は高頻度に起こる | オリジナルの細胞では検出されない CNV が iPS 細胞で検出される | ES 細胞と iPS 細胞で異なる CG-メチル化領域のうち iPS 細胞に特異的な割合が 51～56％ |
| 検出方法 | たんぱく質をコードする領域の全ゲノムシークエンス | affymetrix<br>genome-wide human<br>SNP array 6.0 | バイサルファイト・ショットガン・シークエンス法 |
| 原因 | 少数の線維芽細胞に既存の変異<br>リプログラミング<br>培養 | リプログラミング<br>培養 | リプログラミング<br>培養 |

## II. *in vivo* での iPS 細胞の活用

### I. 移　植

ファンコニ貧血の論文[13]では，患者から採取した線維芽細胞の遺伝子変異を修復することで iPS 細胞の樹立が可能となったことが示された．このことは，患者自身から採取した細胞を *in vitro* で治療した後，患者に移植するという自家移植の可能性を強く支持するものである．

現在，iPS 細胞を使用したヒトへの移植治療や治験はまだ行われていない．しかしながら，ES 細胞由来の細胞をヒトに移植する治験や，iPS 細胞由来の細胞を動物モデルに移植する研究が進められている．

米国では，ES 細胞由来の網膜色素上皮細胞を用いたスターガルト病・加齢性黄斑変性治療が第一相臨床治験として開始されている[14]．また，移植を目的とし，ヒト iPS 細胞から網膜色素上皮および視細胞が異種成分不含の分化方法により誘導されている[15]．ブタ iPS 細胞由来の視細胞を，ヨード酢酸塩投与により視細胞を除去したブタに移植し，移植した視細胞が外顆粒層に生着することが示された[16]．

パーキンソン病ではカニクイザルの ES 細胞から分化誘導したドーパミン作動性神経細胞をパーキンソン病モデルのカニクイザルの脳に移植し，移植された細胞が脳内で生着し神経症状の改善をもたらすことが報告されている[17]．ヒト iPS 細胞を用いた研究では，分化誘導したドーパミン作動性神経細胞をパーキンソン病モデルのラットに移植し，運動疾患が改善されることが示された[18]．

脊髄損傷や筋萎縮性側索硬化症（amyotrophic lateral sclerosis: ALS）モデルでは主としてグリア細胞が移植実験に利用されている[19]．脊髄損傷モデルではヒト ES 細胞から分化誘導したオリゴデンドロサイトを脊髄損傷モデルのラットに移植し行動が改善されることを示している[20]．グリア前駆細胞を ALS モデルマウスに移植した実験では，正常なアストロサイトの運動ニューロン保護効果が示唆されている[21]．

### II. 今後の課題

iPS 細胞由来細胞の移植については，未分化な細胞から生じる奇形腫や移植する目的細胞が過剰に増殖するといった腫瘍形成の問題がある．これらの問題を回避するために未分化細胞の除去や目的細胞の *in vivo* での長期にわたる観察が必要である．

## おわりに

近年，中枢神経系の治療に対する研究は，神経幹細胞・iPS 細胞・ES 細胞からの神経細胞の発生・分化に基づき進められてきた．一方で，神経細胞が機能を有するためには，神経細胞の機能再生も重要であり，軸索伸長に関する研究が重ねられてきている[22, 23]．軸索伸長には微小管やアクチンフィラメントといった細胞骨格の再構築が必須で，それらを制御しているシグナル伝達経路として，低分子量 G タンパク質 Rho ファミリー（Rho, Rac および Cdc42）があげられる．神経細胞において，Rho の活性化は神経突起の退縮に必要であり，Rac と Cdc42 はそれぞれ成長円錐における葉状仮足および糸状仮足形成を制御し，神経突起の伸長に必要であることがわかっている[24]．さらに，Rho/ROCK 経路は神経細胞のアポトーシスに関与しており，ROCK 阻害剤である Y-27632 を移植細胞に加えることにより移植細胞である神経幹細胞のアポトーシスが抑制されることが示されている[25]．以上のことから，神経細胞またはグリア細胞の移植時に低分子量 G タンパク質 Rho ファミリーに作用する薬剤を使用することで移植細胞の生存率を上げ，神経細胞の突起伸長を促すことなどが期待される．これまでの中枢神経の研究を統合した手法により，神経系の再生医療においてさらなる発展が期待される．

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Review

# Neurodegenerative disease-specific induced pluripotent stem cell research

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ABSTRACT

Neurodegenerative disease-specific induced pluripotent stem cell (iPSC) research contributes to the following 3 areas; "Disease modeling", "Disease material" and "Disease therapy".
"Disease modeling", by recapitulating the disease phenotype *in vitro*, will reveal the pathomechanisms. Neurodegenerative disease-specific iPSC-derived non-neuronal cells harboring disease-causative protein(s), which play critical roles in neurodegeneration including motor neuron degeneration in amyotrophic lateral sclerosis, could be "Disease material", the target cell(s) for drug screening. These differentiated cells also could be used for "Disease therapy", an autologous cellular replacement/neuroprotection strategy, for patients with neurodegenerative disease.
Further progress in these areas of research can be made for currently incurable neurodegenerative diseases.



## Contents



Neurodegenerative diseases are caused by the degeneration of selected neurons: cortical neurons in Alzheimers' disease, dementia with Lewy bodies, or frontotemporal lobar degeneration, midbrain dopaminergic neurons in Parkinson's disease, cerebellar neurons in spinocerebellar degeneration, and upper and lower motor neurons in amyotrophic lateral sclerosis (ALS). It is widely believed that neurodegenerative diseases generally arise through the same process; neuronal dysfunction [1], the gradual accumulation of misfolded protein and the acceleration of aggregate formation [2], neuronal death [3], and disease progression caused by non-neuronal cells [4,5].

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Neurodegenerative diseases are still intractable, although studies using molecular biology continue to enhance our understanding of neurodegeneration.

ALS, one of the neurodegenerative diseases, is characterized by the degeneration of upper and lower motor neurons, leading to fatal paralysis. The name "ALS" originated from the pathological observation that a distinct myelin pallor in the lateral part of the spinal cord represents degeneration and loss of the axons of upper motor neurons in the spinal cord [6,7]. This relentless disease is characterized by the degeneration of somatic motor neurons in the spinal cord, brain stem, and cortex. Common symptoms are progressive muscular atrophy, difficulty in swallowing and speech, and respiratory failure. Generally, the disease has a midlife onset; it is found in the 45–60 year age group and the typical disease course is 1 to 5 years. The proportion of affected individuals in the population is 4 to 6 per 100,000, and the lifetime risk is about 1 in 1000 [3,6–8].

Approximately 10% of patients with ALS are inherited (familial ALS, FALS), while the remaining have no family history of ALS (sporadic ALS, SALS) [4–8]. After the missense mutations in the gene that encodes the antioxidant enzyme Cu/Zn superoxide dismutase 1 (SOD1) was found in 10–20% of patients with FALS in 1993, most studies in the field have focused on revealing the mechanism of SOD1-mediated motor neuron degeneration [4–8]. SOD1 changes superoxide radicals into oxygen and hydrogen peroxide. When mutated SOD1 are overexpressed in rodents, this causes phenotypes of an ALS-like motor neuron disease, which is not rescued by overexpression of wild-type SOD1. These observations suggest that a toxic gain of function mechanisms, but neither loss of function nor haploinsufficiency, cause mutant SOD1-mediated FALS. Genetically engineered mutant SOD1 animal models have provided abundant information regarding the possible mechanisms of this disease [4–8].

Several therapies with drugs have shown therapeutic effects in SOD1 transgenic rodents, but clinical trials in humans have not been successful. This suggests that current ALS rodent models can provide only limited or extra insight into the pathogenesis of human ALS. Therefore, for drug screenings, human resources, which possess human molecular signaling pathways different from rodents, are supposed to be required. Human resources could be robustly supplied by differentiating human stem cells, especially neurodegenerative disease-specific induced pluripotent stem cells (iPSCs), which are generated by reprogramming adult fibroblast cells of neurodegenerative disease including ALS [9] by using forced expression of the transcription factors—Klf-4, Sox-2, Oct-4, and c-Myc. The iPSCs have the same advantages as traditional stem cells due to their ability to generate differentiated cells such as neurons and glia from individuals [10–12].

Disease-specific iPSCs [13–21] research is a new field that could contribute to the following 3 areas; "Disease modeling", "Disease material" and "Disease therapy" (Fig. 1).

## Disease modeling

"Disease modeling" by recapitulating the diseases phenotype *in vitro* would make it possible to study how different cell types are involved in the pathobiology of neurodegenerative diseases, and to unravel the cellular mechanisms that may trigger familial, as well as sporadic, forms of the disease. In an ALS study, Dimos et al. successfully directed the differentiation of iPSCs, generated from an elderly patient with FALS and a SOD1 mutation, into motor neurons expressing appropriate motor neuron markers including Hb9 and ISLET [9]. While both spinal muscular atrophy (SMA) [13] and ALS [9] are neurodegenerative diseases of motor neurons, only SMA motor neurons from disease-specific iPSCs show phenotypes. This may be due to the fact that the onset age of ALS is after middle age while most SMA cases begin in childhood. Furthermore, the iPSC-derived neurodegenerative models that have been developed for SMA and familial dysautonomia (FDA) [17] take the autosomal recessive inherited form. In neurodegenerative disease modeling from iPSCs, genetic information, the environment, or senescence all contribute to neurodegeneration, and therefore it would be critical to promote these conditions *in vitro*. These studies have led to new avenues for drug development for neurodegenerative diseases.

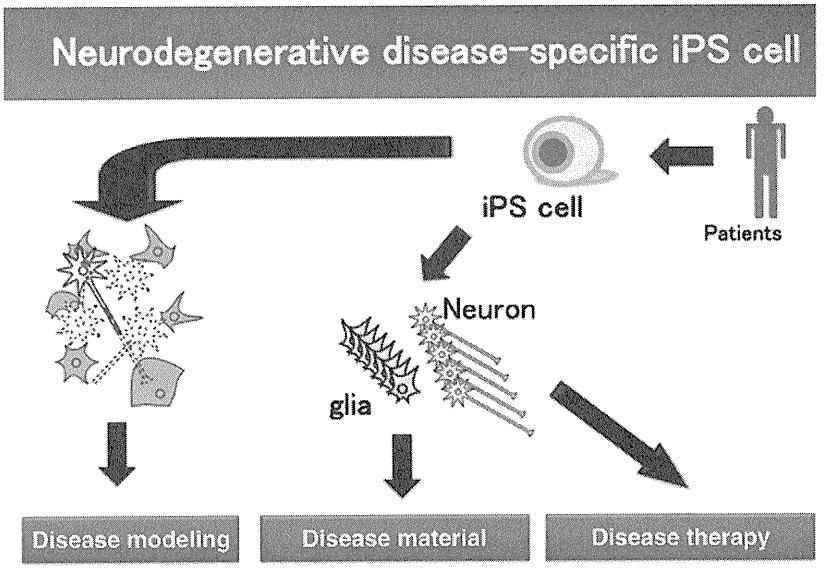


**Fig. 1 – Neurodegenerative disease-specific iPSC research contributes to the following 3 areas; "Disease modeling", "Disease material" and "Disease therapy".**

## Disease material

Affected cells in neurodegenerative disease(s) as "Disease material", which cannot be collected from patients, can be generated from disease-specific iPSCs. These cells possess the genetic information of the patient. Non-neuronal cells, including glial cells [4,5], can be a target of neurodegenerative disease-specific iPSC research based on non-cell autonomous neurodegeneration hypothesis demonstrated by following studies.

The absence of motor neuron degeneration with the synthesis of mutant SOD1, under the control of neuron-specific promoter, provided the initial supportive evidence that the disease probably does not arise from damages caused within motor neurons through cell-autonomous mechanisms [22]. The onset of the disease was delayed and the survival period was extended when part of mutant SOD1 in motor neurons was removed using the Cre–loxP system [23], although, after the disease onset, the period of disease progression was almost unaffected. Similarly, virus-mediated small interfering RNA knock-down, within which the CNS selectively suppressed mutant SOD1 in motor neurons, showed a robust delay in onset [24–26]. On the other hand, based on the results of further studies on such transgenic animals, disease progression is determined by the amount of mutant SOD1 in astrocyte/microglia [23,27]. This suggests that the amount of mutant SOD1 in motor neurons (cell autonomous) is relevant to disease initiation, while progression is dependent on non-neuronal cells, including astrocytes and microglia (non-cell-autonomous).

A chimeric mice study that included a combination of wild-type cells and cells expressing mutant SOD1 directly showed an evidence for a non-cell-autonomous disease mechanism [28]. Motor neurons surrounded by wild-type neighbors survived longer with no degeneration despite mutant SOD1 expression, while motor neurons that were genetically normal were damaged by neighboring mutant SOD1-expressing cells. These results support the idea that non-cell autonomous mechanisms contribute to neurodegeneration in mutant SOD1-mediated ALS.

Embryonic stem cell (ESC) research [30–33] also contributes to our understanding of non-cell-autonomous mechanisms in neurodegenerative disease. It has been demonstrated that mutant SOD1-expressing astrocytes diminish the survival of motor neurons over a 2-week period compared to normal glial cells by the co-culture of primary, mutant SOD1-expressing astrocytes with primary motor neurons purified from embryos, or with motor neurons generated by the differentiation of mouse embryonic stem cells. Astrocyte-derived toxicity, which is transferred by astrocyte conditioned media, acts on wild-type as well as mutant-SOD1 motor neurons, and is specific to motor neurons with no effect on sensory neurons or interneurons [29,30]. Studies also showed that human ESC-derived motor neurons are also sensitive to the toxic effect of glial cells carrying an ALS-causing mutation, which supports previous suggestions that non-neuronal cells contribute to the pathogenesis of ALS [31,32]. Astrocytes expressing ALS-linked mutated SOD1 release factors that are toxic to motor neurons. Further studies may show the relevance to these factors with the reduced expression of GLT-1 that is commonly seen in human ALS [33]. These experiments have provided an *in vitro* paradigm for the use of stem cell-derived co-culture experiments in exploring cell–cell interactions in ALS or in other neurodegenerative diseases.

In addition to the SOD1-mediated ALS studies, there are supporting evidence showing non-cell autonomous neurodegeneration in spinocerebellar ataxias (SCA), which is one of neurodegenerative diseases with characteristics of cerebellar neurodegeneration that lead to progressive motor incoordination [5]. The most affected cells are cerebellar Purkinje neurons. Non-neuronal neighbors to these neurons are Bergmann glia, cerebellar-specific astrocytes, with long finger-like processes to enwrap the dendritic trees of Purkinje cells [34]. SCA7 is caused by polyQ expansion in the gene encoding ataxin-7. Transgenic mice that showed mutant polyQ expression only in Bergmann glia and other astrocytes by using the GFAP promoter would be sufficient to cause Purkinje cell degeneration. Moreover, the Purkinje cell degeneration was remarkably similar to that induced by polyQ expansion under the control of the Prion promoter, both neuronal and glial promoters, demonstrating non-cell-autonomous Purkinje cell degeneration [5,35].

These findings suggest that disease-causative protein in glial cells could be target molecule(s) in targets cell(s) in drug screening by using disease-specific iPSCs. Although it is still unclear whether glial cells in patients elicit the same toxicity as ALS model astrocytes, as the latter commonly harbor multiple copies of mutant SOD1 [36]. An analysis of iPSC-derived glial cells from patients with mutant SOD1-induced ALS could possibly reveal whether a single gene copy renders human glial cells as toxic as those harboring multiple mutant SOD1 copies [36]. As a consequence, it is critical to analyze the mechanisms for disease pathways and to perform drug screening by using human resources derived from neurodegenerative disease-specific iPSCs followed by evaluating drug delivery, optimal safe dose or time window in animal model studies.

## Disease therapy

There is more than one method for generating iPSCs (i.e., retrovirus, lentivirus, adenovirus, plasmid vector, small compounds, protein transduction etc.) [37] from multiple origins. The iPSCs also could provide an autologous cellular replacement/neuroprotection strategy for patients with neurodegenerative diseases [36], "Disease therapy". In addition to transplantation of specific neurons from iPSCs for replacement therapy, transplantation of glial cells from iPSCs can be used for neuroprotection [36]. Before the transplantation approach can be applied clinically, numerous hurdles must be overcome. For these putative stem cell-based therapies, we must first identify the optimal cell dose and source, and/or the route of delivering the cells [36]. Transplantation therapies would consist of the delivery of a combination of subtypes of neuronal cells to provide both cellular replacement and neuroprotection.

Further progress in these areas of research can be developed for currently incurable neurodegenerative diseases.

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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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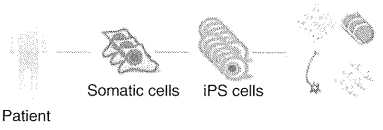


**Figure 1** Generation of induced pluripotent stem cells (iPSCs) and their differentiation potential. iPSCs are derived from easily accessible somatic cells. In contrast to organ-specific stem cells, pluripotent stem cells such as embryonic stem cells and iPSCs show the ability to differentiate into 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<br>2. Decreased SMN protein level (evaluated with WB/IA)<br>3. Rescue phenotype 2 with 1 mmol/l valproic acid |
| Familial dysautonomia[42] (*IKBKAP*) | AR | Infancy | 1. Increased abnormal splicing in differentiated neural crest<br>2. Decreased no. (%) of ASCL1+, Tuj1+ neurons<br>3. Migratory dysfunction (scratch assay)<br>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)<br>2. Differentiate into CD34+/hematopoietic progenitors |
| Dyskeratosis congenita[44] (*XR: DKC1*) | XR (AR/AD) | Adolescence | 1. Elongated telomere in iPSCs (TERT/TERC↑)<br>2. Shortened telomere after differentiation (TERT/TERC↓) |
| LEOPARD syndrome[45] (*PTPN11, RAF1, SHOC2*) | AD | Infancy to adolescence | 1. Enlarged cell size of differentiated cardiomyocyte<br>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)<br>2. Reduced RTT protein level/cell size and rescue by gentamicin (100 µg/ml) at Q244X clone<br>3. Reduced activity-dependent calcium transients<br>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]

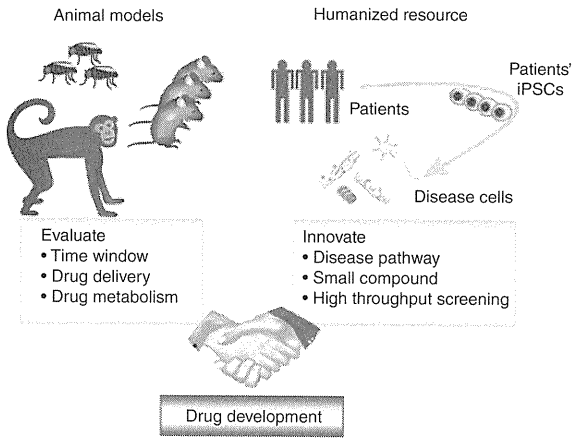


**Figure 2** Combined approach involving animal models and induced pluripotent stem cell (iPSC) technology. The new iPSC technology is complemented by a drug development strategy in preclinical settings that uses animal models and other conventional approaches.

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.

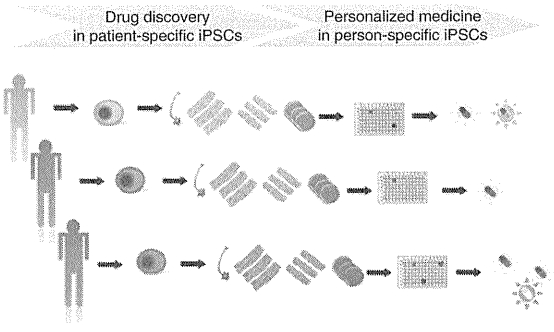


**Figure 3** Personalized medicine based on induced pluripotent stem cell (iPSC) technology. iPSC technology is highly amenable to individualized approaches. Person-specific iPSCs can be derived, differentiated into specific cell types, and used for therapeutic/toxicity response assays.

### 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

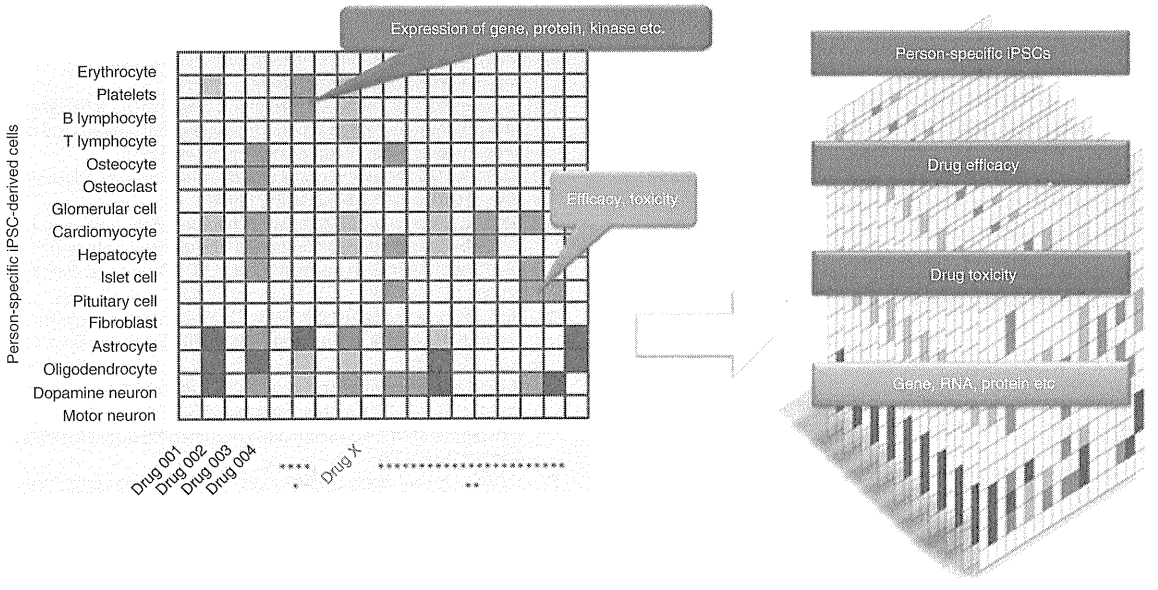


**Figure 4** "Pharmaco-iPSCellomics" by person-specific iPSCs. iPSCs derived from individual subjects/patients can be differentiated into multiple cell types, thereby providing a personalized iPS-cellome platform. This cell-based system can be used for drug discovery and selection of clinical therapeutics 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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# 4）iPS細胞を用いた神経・精神疾患モデル研究

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**key words**　iPS cell, disease modeling, drug screening, neurodegenerative disease, psychiatric disease

**要　旨**

人工多能性幹細胞 induced pluripotent stem cell (iPS細胞) は，体細胞に胚性幹細胞 embryonic stem cell（ES細胞）で発現している数種類の遺伝子を導入することにより作製され，ES細胞と同様に様々な系譜の細胞に分化させることができる．iPS細胞作製技術の開発により，患者自身の遺伝子情報を有した疾患標的細胞の作製が可能となった．また，原因遺伝子が同定されている疾患だけでなく，遺伝歴のない孤発性疾患においても疾患標的細胞の作製が可能である．本稿では，2007年にヒトiPS細胞作製が可能となってから現在までのiPS細胞を用いた神経・精神疾患モデル研究について述べる．

**動　向**

中枢神経は体の深部に位置すること，再生が難しいことなどから，ヒトにおける神経疾患の直接的な病態の解析は困難である．そのため，神経・精神疾患の研究では動物モデルや細胞モデルによる間接的な解析が中心である．近年，様々な遺伝子操作された疾患モデル動物が作製され，神経・精神疾患の病態解明が急速に進んでいる．しかし，疾患モデル動物で有効であると証明された薬剤がヒトにおける臨床研究で有効性を認めないことも少なくはない[1]．その原因として，ヒトとモデル動物の遺伝子発現の違いや解剖学的な違いが関与していることなどが考えられる[2]．

2006年にマウス[3]，2007年にヒト[4]人工多能性幹細胞 induced pluripotent stem cell (iPS細胞）が作製された．ヒトiPS細胞は，胚性幹細胞 embryonic stem cell (ES細胞) で発現している4つの転写因子 (Oct3/4, Sox2, Klf4, c-Myc) をレトロウイルスベクターを用いてヒト線維芽細胞に導入することで体細胞を初期化し（リプログラミング），多能性幹細胞が樹立された．iPS細胞はES細胞に匹敵する多能性を獲得した多能性幹細胞であり，ほぼ無限に増殖し，内胚葉・中胚葉・外胚葉への分化能力を有している．移植後に腫瘍化がみられることがあるため，近年では，より腫瘍化しにくいiPS細胞の樹立方法が開発されている[5,6]．

iPS細胞作製技術の開発により，患者自身の体細胞からiPS細胞を樹立することで，患者の遺伝子情報を有した神経細胞の作製が可能となった．iPS細胞はすでに原因遺伝子が同定された疾患だけでなく，原因遺伝子が明らかにされていない疾患においてもモデル細胞となり得る利点がある．

## A. 疾患特異的iPS細胞の樹立と疾患モデルの作製

iPS細胞作製技術を用いて，様々な神経・精神疾患患者の体細胞から疾患iPS細胞が作製されている（表1）．iPS細胞を樹立後，疾患標的細胞への分化誘導が行われる．例えば，筋萎縮性側索硬化症 amyotrophic lateral sclerosis（ALS）患者由来iPS細胞では，retinoic acid，sonic hedgehogの添加により神経前駆細胞から脊髄運動神経細胞が誘導される[7]．また，パーキンソン病患者由来iPS細胞からは，sonic hedgehog，fibroblast growth factor（FGF）8の添加によりドパミン神経細胞が誘導されている[8]．現在，iPS細胞が作製されている主な神経・精神疾患を以下に記載する．

### 1．パーキンソン病（Parkinson's Disease）

2009年にSoldnerらは50歳代から80歳代の5人の孤発性パーキンソン病患者の線維芽細胞より

**表1　主な神経・精神疾患患者由来iPS細胞を用いた論文報告**

| 疾患 | 遺伝形式 | 遺伝子異常 | 再現された表現型 | reference |
|---|---|---|---|---|
| パーキンソン病 | 孤発性 | - | - | Soldner 2009[8] |
| パーキンソン病 | 常染色体劣性遺伝 | *LRRK* (G2019S) | 酸化ストレス下でcaspase-3の増加，ドパミン神経細胞死の増加 | Nguyen 2011[10] |
| パーキンソン病 | 常染色体劣性遺伝 | *PINK1* (Q456X, V170G) | ストレス下でドパミン神経細胞におけるParkin蛋白のミトコンドリア移動の障害 | Seibler 2011[11] |
| 筋萎縮性側索硬化症 | 常染色体優性遺伝 | *SOD1* (L144F) | - | Dimos 2008[7] |
| 筋萎縮性側索硬化症 | 常染色体優性遺伝 | *SOD1* (L144F, G85S) | - | Boulting 2011[12] |
| 脊髄性筋萎縮症1型 | 常染色体劣性遺伝 | *SMN1* deletion | 運動神経細胞の減少，細胞体の小型化，SMN蛋白の発現量低下 | Ebert 2009[13] |
| ハンチントン病 | 常染色体優性遺伝 | *IT15* (72CAG repeats) | - | Park 2008[14] |
| フリードライヒ運動失調症 | 常染色体劣性遺伝 | *FXN* (GAA expansion) | - | Ku 2010[15]<br>Liu 2011[16] |
| 家族性自律神経失調症 | 常染色体劣性遺伝 | *IKBKAP* (homozygous 2507+6T＞C) | スプライシング異常，神経細胞数の減少および遊走能の低下 | Lee 2009[17] |
| 統合失調症 | 孤発性 | *DISC1* (4bp deletion at the exon-intron 12 region) | - | Chiang 2011[18] |
| 統合失調症 | 孤発性 | - | synaptic connectivityの低下 | Brennand 2011[19] |
| レット症候群 | X染色体優性遺伝 | *MeCP2* (1155 del32; Q244X) | 神経細胞体の小型化，シナプス数の減少，電気生理学的機能の異常 | Marchetto 2010[20] |
| レット症候群 | X染色体優性遺伝 | *MeCP2* (Δ exon 3-4; T158M) ) | 神経細胞体の小型化 | Cheung 2011[21] |

iPS細胞を樹立した[8]．2011年，Nguyenらは遺伝性パーキンソン病の原因遺伝子のひとつである*Leucine Rich Repeat Kinase-2*（*LRRK2*）遺伝子にG201S変異を有する患者からiPS細胞を作製し，ドパミン神経細胞に分化させた．これまでのパーキンソン病研究から，変異*LRRK2*の過剰発現によりα-synucleinが蓄積することが示されている[9]．線維芽細胞やiPS細胞にはα-synucleinの蓄積はみられなかったが，神経細胞に分化誘導することにより，細胞内にα-synuclein蛋白の蓄積を生じた．また，*LRRK2*遺伝子異常を有する患者由来iPS細胞から分化させた神経細胞では，酸化ストレス関連遺伝子の発現増加がみられた．さらに，hydrogen peroxide, MG132, 6-hydroxydopamineによるストレスに対して，caspase-3の活性化や細胞死がコントロールよりも有意に惹起された[10]．

Seiblerらは，*PTEN-induced putative kinase 1*（*PINK1*）遺伝子異常を有する遺伝性パーキンソン病患者3人からiPS細胞を作製した．これらのiPS細胞をドパミン神経細胞に分化させたところ，ストレス下でのParkin蛋白のミトコンドリアへの輸送の障害がみられた．この所見はレンチウイルスでPINK1蛋白を過剰発現することにより改善した[11]．

## 2. ALS

Dimosらは，遺伝性ALSの原因遺伝子のひとつである*SOD1*遺伝子のL144F変異を有する82歳と89歳の高齢患者の線維芽細胞からiPS細胞を作製し，下位運動神経細胞のマーカーであるHB9およびISLET1/2陽性を示す運動神経細胞へ分化誘導を行った[7]．Boultingらは，*SOD1*遺伝子のG85S変異，L144F変異を有する患者由来のiPS細胞を作製した．運動神経細胞へ分化誘導し，免疫染色法および電気生理学的手法を用いて，作製した運動神経細胞の評価を行っている[12]．しかし，現時点ではALSにおけるiPS細胞を用いた病態再現は報告されていない．

## 3. 脊髄性筋萎縮症 spinal muscular atrophy (SMA)

SMAは下位運動神経細胞が障害される疾患で，乳児期に発症する重症型のtype1，中間型のtype2，18カ月から思春期の間に発症する軽症型のtype3がある．Ebertらは，3歳のtype1患者由来のiPS細胞を作製し，脊髄運動神経細胞に分化させた．コントロールに比較して脊髄運動神経細胞数の減少と細胞体の小型化がみられた．また，SMN蛋白の発現低下がみられた．このSMN蛋白の減少は，histone deacetylase阻害薬であるvalproic acidやtobramycinによって改善した[13]．

## 4. ハンチントン病 Huntington disease

Parkらは，*IT15*遺伝子に伸長CAG repeatを有する20歳の患者からiPS細胞を樹立し，作製したiPS細胞におけるCAG repeatの伸長を示した[14]．

## 5. 脊髄小脳変性症 spinocerebellar degeneration

脊髄小脳変性症のひとつである，フリードライヒ運動失調症患者由来のiPS細胞が作製されている．フリードライヒ運動失調症は常染色体劣性遺伝を呈し，神経変性と心筋障害などを呈する．原因遺伝子*frataxin*のイントロンのGAAリピート伸長がみられる．Kuらは，フリードライヒ運動失調症患者由来iPS細胞を樹立したところ，クローンによるCAGリピートの不安定性がみられた[15]．Liuらは，2人の患者からiPS細胞を作製した．作製されたiPS細胞では，GAAリピートの伸長とfrataxin mRNAの減少を認めた[16]．

### 6. 家族性自律神経失調症 familial dysautonomia

家族性自律神経失調症は*I-κ-B kinase complex-associated protein* (*IKBKAP*) 遺伝子の変異によって末梢神経障害を生じる稀な疾患である. Leeらは, 10歳の家族性自律神経失調症患者由来のiPS細胞を作製した. このiPS細胞から分化させた神経堤細胞においてスプライシングの異常を認めた. また, 自律神経細胞の数の低下や遊走能の低下が認められた. 一方, これらの所見は植物ホルモンの一種であるkinetin投与により改善した[17].

### 7. 精神疾患

Chiangらは, 統合失調症やうつ病などの精神疾患発症の危険因子と考えられている*Disrupted-in-Schizophrenia 1* (*DIS1*) 遺伝子の変異を有する統合失調症患者からiPS細胞を樹立した[18]. Brennandらは, 4人の統合失調症患者由来のiPS細胞を樹立し, 神経細胞に分化させた. 統合失調症の患者由来iPS細胞から分化誘導した神経細胞は, コントロールに比較して, 神経突起数の減少, シナプス蛋白の減少を呈し, synaptic connectivityの低下を示した. これらの所見は, 抗精神病薬のひとつであるloxapineによって改善がみられた[19].

メチル化制御に関連する遺伝子*MeCP2*の変異で生じるレット症候群患者のiPS細胞由来神経細胞は, 神経細胞体の小型化がみられた[20,21].

## B. iPS細胞を用いた神経・精神疾患モデル研究の課題と今後の展望

iPS細胞を用いた疾患モデリングにおいて, いくつかの克服すべき点がある. iPS細胞では同じ患者体細胞由来であっても分化誘導効率がクローンによって異なる場合がある. ES細胞でも同様のクローンによる分化能の違いが報告されている[22]. 疾患解析で生じた差がクローン間の差であるのか, 個体間あるいは疾患による差であるのか, 慎重に解析する必要がある. よって, iPS細胞を用いた疾患モデル研究では, クローン間のばらつきを超えた病態再現が必要となる. また全ゲノム関連解析 genome wide association studies (GWAS) などにより, 疾患関連single nucleotide polymorphisms (SNPs) を全く有さない人がいることは考えにくく, 疾患に応じてコントロールの設定を選択する必要がある[2].

多くの神経・精神疾患, 特に神経変性疾患は比較的高齢になってから発症すること, 孤発性疾患の場合には遺伝的背景以外の環境要因が発症に関連していることから, 神経変性疾患の病態再現を行うには, 加齢変化を短縮させ, 環境要因と関連する因子の導入を行う必要があるかもしれない.

### むすび

近年, 遺伝子変異を有する神経・精神疾患の病態解明の急速な進歩により, 神経・精神疾患の知見が深まってきている. iPS細胞を用いた研究の重要な特徴は, 原因遺伝子が同定されている疾患だけでなく, 原因遺伝子が不明な遺伝性疾患や孤発性疾患における病態解明や創薬への応用が期待できる点である. 神経・精神疾患患者の多くは孤発性であり, iPS細胞を用いた神経・精神疾患病態解明への研究の発展と一日も早い治療開発への応用が望まれる.

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