により数多くのクローン数のヒトiPS細胞が樹立されていると予測され，幹細胞の未分化性維持・遺伝子発現特性の解析，さらには癌研究など広範な研究領域に大きなインパクトを与えている．

本邦においても，ヒト幹細胞を用いる臨床研究に関する指針が2010年に改訂され，ヒトiPS細胞の臨床研究が法的に認可された(ES細胞は2011年時点で禁止されている)．

神経疾患への応用を考えた場合，ヒトiPS細胞の神経分化誘導には，ヒトES細胞で培われた分化誘導法の適応が多くの場合応用可能である[17]点は大きな利点となりうる．そしてiPS細胞を用いた再生医療は，ES細胞樹立で議論される倫理的問題点が少なく，患者個々人から樹立したiPS細胞を用いることで拒絶反応を避けられる可能性もある．しかしながら，iPS細胞の安全性確認にかかる時間・コスト等についてはES細胞と同様に克服すべき課題を有する．

多能性幹細胞と造腫瘍性

さらに，iPS細胞・ES細胞に共通の課題として造腫瘍性がある．造腫瘍性には大きく分けて，未分化細胞から生じる奇形腫と，ある程度分化した細胞から生ずる腫瘍性増殖の可能性がある．

前者は，分化抵抗性のある未分化細胞が移植片に混入することが問題とされている[18]．未分化細胞に特異的な表面マーカーや遺伝子発現パターンを用いることで，この点は危険性を低減できると予測される．

一方で後者のある程度分化した細胞の腫瘍性増殖については，免疫不全動物における長期観察など前述のES細胞臨床治験を経た綿密な安全性確認が必要になるであろう．

今後の展望

これらの点を克服するため，本邦においてはHLA-A, B, DRの3遺伝子座についてホモ型であるドナー由来のiPS細胞株を50株作成し，バンク化しておくことで，将来的な移植治療を見据えたとき日本人の90.7%をカバーできることが報告されている[19]．

このバンク化されたiPS細胞の安全性をあらかじめ十分確認しておくことで，比較的病状進行の速いALSにおいても，安全性が高く拒絶反応の少ない細胞移植治療を速やかに実施できることが将来的に期待される．

むすび

体性幹細胞に端を発したALSの治療研究は，さらなる安全性の確認が必須であるが少しずつ進歩している．また多能性幹細胞を用いたALSの再生医療も，現時点では克服すべき課題が多い．しかしながら，ES細胞を用いた臨床研究が既に始まり，その課題を克服するべくiPS細胞の臨床応用にも道筋がつけられつつある現在，ALSの神経再生医療実現化に向けた着実な進歩を期待したい．

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Chapter V

Induced Pluripotent Stem Cell Technology for the Study of Neurodegenerative Diseases

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Abstract

Induced pluripotent stem (iPS) cell technology has paved new ways for disease modeling and drug discovery. Disease modeling with the differentiated neuronal cells from patient-specific iPS cells partially recapitulated the phenotypes of spinal muscular atrophy (SMA), familial dysautonomia (FD) and Rett syndrome. Furthermore, proof of the efficacy of candidate drugs by iPS cell-based assay on SMA and FD has been reported.

There are several obstacles for disease modeling using iPS cell-derived neuronal cells. First, differentiated neuronal cells from patient-specific iPS cells might not be provoked sufficiently toward senescence to manifest the phenotype of late-onset diseases such as Parkinson disease (PD) and amyotrophic lateral sclerosis (ALS). Second, there are heterogeneous populations in cultured cells differentiated from iPS cells that might affect the disease phenotype. The propensity of various differentiations among iPS cells leads to the heterogeneity (Figure 1).

In this review, we will describe recent literature concerning the application of iPS cell technology for the study of neurological diseases and also discuss some experimental requirements.

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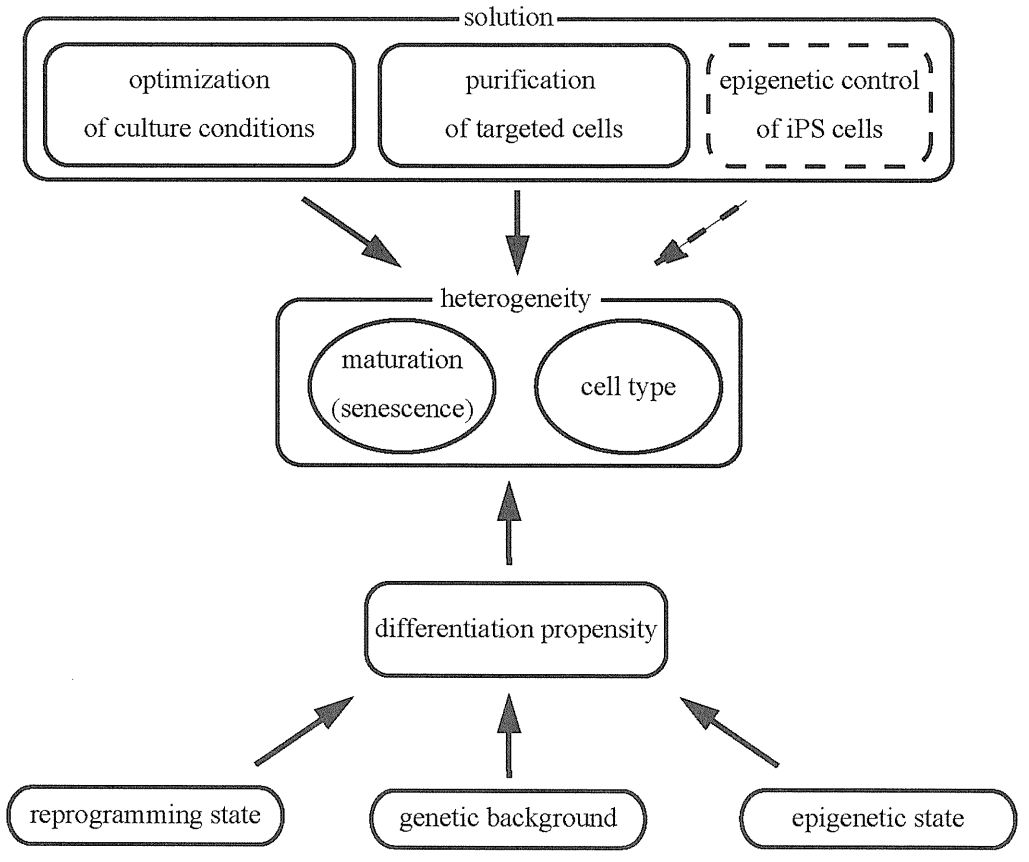

Figure 1. Heterogeneity, including maturation stage and cell population, is a result of the variety of differentiation propensities of iPS cells caused by reprogramming state, genetic background and epigenetic state. Purification of targeted cells and optimization of culture conditions will reduce the heterogeneity and lead to progresses in iPS cell technology.

Introduction

iPS cells are generated by transduction of transcription factors, which are expressed in embryonic stem (ES) cells, in somatic cells (Takahashi *et al.*, 2006; Takahashi *et al.*, 2007; Yu *et al.*, 2007). iPS cells are morphologically identical to ES cells and have the ability to self-renew and differentiate into cells of all germ layers. iPS cell technology makes it possible to analyze *in vitro* affected cell populations that are differentiated from disease-specific iPS cells. This then should enable us to determine the underlying mechanisms of disease, screen new drugs, and develop cell therapy.

Disease phenotypes were partially recapitulated in the differentiated cells from patient-specific iPS cells in some diseases such as spinal muscular atrophy (SMA) (Ebert *et al.*, 2009), familial dysautonomia (FD) (Lee *et al.*, 2009) and Rett syndrome (Marchetto *et al.*, 2010). Furthermore firm evidence of the efficacy of some candidate drugs for SMA (Hastings *et al.*, 2009) and FD (Lee *et al.*, 2009) has also been presented. Other disease phenotypes such as Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) have not yet been described in such terms, although midbrain dopaminergic neurons (Soldner *et al.*, 2009) and spinal motor neurons were successfully generated from patient-specific iPS cells (Dimos *et al.*, 2008).

Genetically corrected somatic cells from Fanconi anaemia patients can be reprogrammed to iPS cells (Raya *et al.*, 2009). Further, corrected patient-specific iPS cells can give rise to haematopoietic progenitors of myeloid and erythroid lineages whose phenotypes are normal. This demonstrates the potential value for cell therapy application.

In this review, we will describe recent literature featuring iPS cell technology for the study of neurological diseases, and we will discuss experimental requirements for overcoming several obstacles in disease modeling.

Main Document

iPS Cells Derived from Patients with Neurological Diseases

Since the start of the development of iPS cell technology, disease-specific iPS cell lines were generated from individuals with neurological diseases, as summarized in Table 1. Neurological diseases can be roughly divided into two categories. One includes early-onset, neurodevelopmental diseases such as Rett syndrome (Marchetto *et al.*, 2010), Prader-Willi/Angelman syndrome (Chamberlain *et al.*, 2010), Fragile X syndrome (Urbach *et al.*, 2010), SMA, FD, Friedreich's ataxia (Ku *et al.*, 2010) and Down syndrome (Park *et al.*, 2008). The other includes late-onset, neurodegenerative diseases like ALS, PD and Huntington's disease (Park *et al.*, 2008).

A few of the neurodevelopmental diseases caused by single gene abnormalities resulting in highly penetrant phenotypes were successfully recapitulated with iPS cell technology.

SMA is an autosomal recessive genetic disorder caused by mutations in the survival motor neuron 1 gene (SMN1). Mutations in SMN1 significantly reduce SMN protein expression and result in the selective degeneration of lower motor neurons. Ebert *et al.* (2009) showed that SMA patient-derived spinal motor neurons were reduced in number and cell body size at 6 weeks of differentiation compared to the patient's unaffected mother-derived motor neurons. SMN protein is localized in gems, one of the nuclear compartments, and the number of gems present is inversely correlated to disease severity. The authors detected an increased number of SMN gems in nuclei of both fibroblasts and iPS cells derived from the SMA patient. The homozygous loss of SMN1 is partially compensated for by the presence of another gene, SMN2, which also codes for SMN protein. However, the expression of full-length protein generated from SMN2 is substantially lower than that from SMN1. A single nucleotide replacement of SMN2 compared to SMN1 changes splicing, and that transcript encodes truncated protein. Hastings *et al.* (2009) reported that tetracyclines are able to increase the expression of full-length SMN protein from SMN2 by splicing modulation.

Table 1. Disease-specific iPS cell lines.

	Neurological disease	Primarily affected neuronal population	Control	*in vitro* phenotype	Mutated gene in disease-specific iPS cell
early-onset	Familial dysautonomia (FD)	sensory and autonomic neurons	unaffected	impaired migration	IKBKAP
	Fragile X syndrome	global			FMR1 CGG repeat truncation
	Friedreich's ataxia	sensory neurons	SMA-iPS cells, non-disease		FXN GAA·TTC repeat expansion

			unaffected		
	Angelman syndrome	global	unaffected		15q11-q13 paternal deletion
	Prader-Willi syndrome				15q11-q13 maternal deletion UBE3A
	Down syndrome	global			Trisomy 21
	Rett syndrome	global	unaffected	reduced number of synapses	MeCP2
	Spinal muscular atrophy (SMA)	spinal motor neurons	unaffected mother	motor neuron loss	SMN1
late-onset	Amyotrophic lateral sclerosis (ALS)	motor neurons			SOD1
late-onset	Huntington disease (HD)	striatal GABAergic neurons			Huntingtin CAG repeat expansion
late-onset	Parkinson disease (PD)	midbrain dopaminergic neurons			

FD is an autosomal recessive genetic disorder caused by mutations in IKBKAP gene involved in transcriptional elongation and characterized by the degeneration of sensory and autonomic neurons. Lee *et al.* (2009) showed marked defects in neurogenic differentiation and impaired migration of neural crest precursor cells derived from FD patients compared to those from non-affected control. Kinetin, one of the plant hormones, increased normal *IKBKAP* and ameliorated neural differentiation and migration.

Rett syndrome is a progressive neurological disorder caused by mutations in X-linked gene encoding MeCP2 protein. Marchetto *et al.* (2010) reported a reduced number of glutamatergic synapses and alteration of morphology in forebrain neurons derived from Rett syndrome patients compared to those from unaffected controls. At 6 weeks of differentiation, neurons from Rett syndrome patients have a significant decrease in frequency and amplitude of spontaneous postsynaptic currents when compared to those from unaffected individuals.

Obstacles of Modeling Late-Onset Neurodegenerative Diseases

A modeling by iPS technology of polygenic, late-onset neurodegenerative diseases has not been reported. Midbrain dopaminergic neurons and spinal cord motor neurons can be differentiated from PD and ALS patient-specific iPS cells, respectively. Perhaps the disease phenotype may never manifest itself under standard culture conditions because differentiated neurons might be too immature or an environment surrounding neurons might differ between *in vitro* (culture) and *in vivo* (brain or spinal cord). It might be revealed by challenging the neural cells with stressors such as oxygen reactive species, proinflammatory factors or co-culture with another type of cell (Marchetto *et al.*, 2008).

Recent investigations have shown that glial cells are affected by physiological brain aging regardless of the absence of any neurodegenerative pathology (Terry *et al.*, 1987). In astrocytes, advanced age initiates conditions similar to mild reactive gliosis. Astroglial cells from advanced-age brain have higher expressions of glial fibrillary acidic protein (GFAP) and glial calcium-bound protein S100 (Le Prince *et al.*, 1993). Additionally, the number of microglial cells is significantly increased (von Bernhardi *et al.*, 2010).

Moreover, the involvement of these non-neuronal cells in neurodegenerative diseases is increasingly being recognized. AD is characterized by profound neuronal loss and prominent reactive astrogliosis and activation of microglia. As Alois Alzheimer suggested, AD plaques are formed by Aβ deposits, degenerating neurites, astroglial processes and activated

microglial cells (Vehmas *et al.*, 2002). Activated astrocytes and microglia can release proinflammatory factors such as interleukin-1β and prostaglandin E_2 (Mhatre *et al.*, 2004).

Oxidative stress may play an important role in sporadic PD (Zhang *et al.*, 2000), as it is suggested that patient-derived midbrain dopaminergic neurons might be more vulnerable to oxidative stress.

Identification of new and more effective and relevant stressors or environments that mimic senescence or elicit neuronal phenotypes earlier in models of late-onset neurodegenerative diseases will therefore be a critical goal for future research.

Heterogeneous Population of Differentiated Cells

Although differentiation protocols have been reported for human ES cells and iPS cells in which neural stem cells and specific neuronal or glial cell types are enriched, it has in fact been revealed that heterogeneous cell populations exist under these conditions (Chambers *et al.*, 2009; Hu *et al.*, 2009; Lee *et al.*, 2010). Additionally, cells are not able to synchronize the developmental stage of cell populations to the extent seen in normal development *in vivo*, and consequently cells at different stages of maturation are present in such cultures. Naturally, this cellular heterogeneity impedes experimental and clinical utility.

The purification of specialized cells of interest is essential for recapitulating diseases and transplanting cells. Such procedures will rely on our technique of manipulating iPS cells genetically to express selectable markers under the control of cell type-specific promoters that would utilize fluorescent or magnetic cell sorting (Hockemeyer *et al.*, 2009; Placantonakis *et al.*, 2009).

Genomic insertion of reporter gene has been shown to alter gene function (Kustikova *et al.*, 2005). Therefore, identification of unique combinations of cell surface epitopes can facilitate cell therapy because cells of interest are purified without gene manipulation. Pruszak *et al.* (2007 & 2009) identified the combination of cluster of differentiation (CD) surface antigen code for neural lineage and Elkabetz *et al.* (2008) reported Forse 1 as being a marker for neural rosette cells. However, no markers for region-specific postmitotic neurons have so far been identified.

Inhibition of Notch activity by γ-secretase inhibitor resulted in a marked acceleration of differentiation, thereby shortening the time required for the generation of functionally active human ES cell-derived neurons (Borghese *et al.*, 2010). This kind of inhibitor can eliminate proliferating cells from differentiated neurons to reduce the risk of tumor formation after transplantation.

Resolution of this problem may assist in obtaining more robust phenotypes *in vitro*, finding more effective drugs, and supplying safe cells for cell therapy.

A Variety of Differentiation Propensities of iPS Cells

The generation of iPS cells can be accomplished by employing retroviral vectors to overexpress reprogramming factors. After infected somatic cells are fully reprogrammed into iPS cells, the vectors are silenced. Therefore, the reprogramming state may be evaluated by retroviral vector silencing. However, this viral system has been criticized for its permanent integration of exogenous genes into the genome and their possible involvement in differentiation propensity.

Alternatively, transfection of episomal plasmids or modified RNA was also successful in reprogramming somatic cells (Okita *et al.*, 2010; Yu *et al.*, 2009; Yue *et al.*, 2010; Warren *et al.*, 2010). Moreover, the direct delivery of recombinant protein was also reported to reprogram somatic cells (Zhou *et al.*, 2009). Although these methods eliminate the integrated

vector, the gene expression of iPS cells is reflected by that of donor cells (Ghosh *et al.*, 2010). Hence, it is considered that the epigenetic state, including DNA methylation, histone acetylation or histone methylation, may contribute to the differentiation propensities of the respective clones.

Direct conversion from differentiated cells to neurons has been reported (Vierbuchen *et al.*, 2010; Heinrich *et al.*, 2011). This conversion was used to generate mouse subtype-specific neurons from differentiated cells without the need for complete reprogramming to iPS cells. Although this direct conversion is independent from the differentiation propensity of iPS cells, the number of neurons available to study the disease and to be used in high-throughput analysis is limited, and the epigenetic states of these neurons would need to be further analyzed.

Human ES cells are widely variable with regard to epigenetic markers, expression profile and differentiation propensity (Osafune *et al.*, 2008; Rugg-Gunn *et al.*, 2007). And also there is significant intrinsic variance among the iPS cell lines generated to date, as pointed out by Hu *et al.* (2010) & Boulting *et al.* (2011). Pick *et al.* (2009) detected abnormal expression of imprinted genes in a significant number of iPS cell lines. Bock *et al.* (2011) reported that a small set of genes was hypermethylated in iPS cell lines compared to ES cell lines. iPS cells are morphologically similar to ES cells, but the epigenetic state is significantly different between them.

Since there are a variety of methods to generate iPS cells, it is critical for the study of neurodegenerative disease to choose a combination of reprogramming factors, a method of factor delivery, and a cell type to be reprogrammed. For instance, differentiation propensity is partially dependent on the cell type to be reprogrammed (Aoi *et al.*, 2008). For accurate comparison between independent experiments, all conditions should be the same. But there is as yet no standardized parameter for selecting safe and fully reprogrammed iPS cells.

The difficulty of disease modeling is partially attributed to the lack of uniform iPS lines. More robust phenotypes *in vitro* may be obtained in comparison with proper control iPS cell lines.

Conclusion

The development of iPS cell technology has provided both experimental and clinical applications. Once disease-specific phenotypes are identified, this can be translated into cell-based assays for drug screening. However, to accomplish these goals, further improvements are needed. Differentiation methods with higher efficiency will provide larger amounts of cells. The identification of cell surface markers on neuronal or non-neuronal cells will provide homogeneous populations. Highly purified, large amounts of cells of interest will facilitate high-throughput platforms for drug screening. Also crucial is the generation of uniform iPS cells and selection of appropriate iPS cells. This will result in patients having autologous transplantations after their own cells are genetically corrected and/or medicated.

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認知症学 上

—その解明と治療の最新知見—

II. 基　礎　編

認知症研究に用いられる動物・細胞実験モデル　各論

人工多能性幹細胞（iPS細胞）

八幡直樹　　井上治久

II. 基 礎 編

認知症研究に用いられる動物・細胞実験モデル

人工多能性幹細胞(iPS細胞)

Induced pluripotent stem cell

八幡直樹　井上治久

Key words：疾患モデリング，創薬開発

はじめに

マウス線維芽細胞にレトロウイルスを用いて4つの初期化遺伝子を導入することにより，胚性幹(ES)細胞に匹敵する多分化能を有する細胞を樹立(初期化)できることが報告された[1]．この細胞は，人工多能性幹細胞(induced pluripotent stem cell: iPS細胞)と命名され，翌年，ヒト線維芽細胞からiPS細胞が樹立された[2,3]．iPS細胞作製技術によって，体細胞を初期化することにより疾患を有する患者自身の体細胞から，疾患特異的iPS細胞を経て，疾患の標的細胞を入手することが可能になった．iPS細胞を用いることにより神経変性疾患で多くを占める孤発性疾患を解析することが可能になる．また，神経変性疾患においては，神経細胞自身の要因だけでなく，周囲に存在するグリア細胞の神経変性への関与について報告がなされている[4]．ヒトiPS細胞から神経細胞を含めた様々な細胞への分化誘導法の進展に伴い，更なる疾患メカニズムの解明が期待される．

本稿では，iPS細胞を利用した神経変性疾患研究の現状を概説するとともに，神経変性疾患の病態解明，創薬開発へ向けた疾患モデルとしての可能性を述べる．

1. iPS細胞の樹立

ヒトiPS細胞の樹立は，ES細胞で発現している転写因子(Oct3/4，Sox2，Klf4，c-Mycもしくは Oct3/4，Sox2，Nanog，Lin28)をレトロウイルス，レンチウイルスにより皮膚線維芽細胞に導入することで成し遂げられた[2,3]．iPS細胞はES細胞と同様に，胚様体形成による分化誘導を行うことにより，三胚葉すべてへの分化能力をもつ．iPS細胞の利点の一つは，少量の体細胞から作製可能という点であり，更に高齢者の皮膚細胞からの作製例も報告されている[5]．よって，高齢認知症患者からでもiPS細胞の作製が可能である．作製されたiPS細胞は疾患患者の遺伝情報を受け継いでおり，疾患の病態メカニズムの解明に利用できる．

レトロウイルスやレンチウイルスベクターを用いて作製したiPS細胞では導入した遺伝子のmRNAの発現は抑制される(サイレンシング)が，iPS細胞のゲノムには外来遺伝子が挿入される．特に，がん遺伝子であるc-Mycは，当初より再生医療における安全性の問題が懸念されていたが，後にc-Mycを用いない3因子(Oct3/4，Sox2，Klf4)のみ[6]，あるいはc-Mycの代わりにL-Mycを用いた方法[7]によって作製可能であることが報告されている．また，ゲノムへの外来

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表1 神経疾患患者由来iPS細胞を用いた疾患モデリング

疾 患	原因遺伝子	発症年齢	疾患表現型	参考文献
脊髄性筋萎縮症	*SMN1, 2*	乳幼児期～思春期	1. 脊髄運動神経細胞数の低下 2. SMNタンパク質の発現低下 3. 2の表現型をバルプロ酸投与で改善	Ebert AD, et al: Nature 457: 277-280, 2009.
家族性自律神経失調症	*IKBKAP*	乳幼児期	1. 神経堤細胞に分化させた際の異常スプライシング 2. 自律神経細胞数の低下・遊走能の低下 3. 1, 2の表現型をカイネチンで改善	Lee G, et al: Nature 461: 402-406, 2009.
レット症候群	*MeCP2*	乳幼児期	1. グルタミン酸作動性神経シナプス, スパイン数, 細胞体径の減少 2. シナプス減少のIGF1, ゲンタマイシンによる改善 3. 自発的カルシウム移行の減少 4. 自発的後シナプス電流の低下	Marchetto MC, et al: Cell 143: 527-539, 2010.
フリードライヒ失調症	*FXN*	主に若年期	1. iPS細胞における*FXN* GAA・TTCリピートの不安定性 2. 1の表現型をMSH2抑制により改善	Ku S, et al: Cell Stem Cell 7: 631-637,. 2010.
パーキンソン病	*LRRK2*	主に中年期以降	1. α-synuclein蓄積の上昇 2. H_2O_2, 6-OHDA, MG-132投与によりドーパミン作動性神経細胞死の誘導	Nguyen HN, et al: Cell Stem Cell 8: 267-280, 2011.

遺伝子の挿入は疾患特異的iPS細胞の疾患再現に影響を与える可能性はある. この問題を解消する一つの方法としては, ゲノムの挿入されないベクターを用いたiPS細胞を用いる樹立方法[8,9]がある. iPS細胞樹立にあたって利用される体細胞は皮膚線維芽細胞にとどまらず, 様々な組織からの樹立が可能であり[10], 適切な組織を選ぶことにより, より侵襲の少ない操作でiPS細胞が作製できる可能性がある.

2. 神経変性疾患特異的iPS細胞研究の現状

神経変性疾患特異的iPS細胞の樹立に関しては, superoxide dismutase 1(*SOD1*)の変異をもつ筋萎縮性側索硬化症患者皮膚細胞からの作製を皮切りに[5], パーキンソン病[11,12], ハンチントン病[13]など, 幾つかの報告がある.

これら疾患特異的iPS細胞の詳細な性状を正常細胞と比較解析することにより, これまで不明であった疾患の原因, 発症メカニズムが解明される可能性がある. 神経変性疾患特異的iPS細胞を利用し, 疾患再現を示した報告としては, 脊髄性筋萎縮症, 家族性自律神経失調症, レット症候群, フリードライヒ失調症, パーキンソン病患者由来iPS細胞を用いた報告などがある(表1). これらの報告により, 疾患特異的iPS細胞から分化誘導した細胞を使って, 病態を分子生物学, 生化学的解析により評価可能であることが示された. 更には, 疾患特異的iPS細胞が創薬開発のためのスクリーニングに利用可能であることを示している. アルツハイマー病患者由来iPS細胞の樹立に関して学会にて報告されている. 今後の研究の展開が期待される.

3. iPS細胞を用いた創薬開発

モデル動物や細胞株を使った評価系は, ヒトの病態や生理条件を必ずしも反映しておらず, 創薬開発において, 候補薬剤がヒトに対して効果が低い, あるいは副作用が確認されることで開発が中止になることも少なくない. ヒトiPS細胞から分化誘導して得られる細胞を創薬開発や毒性評価などの前臨床試験に利用できれば,

より正確な薬効の確認，毒性評価を行うことができる可能性がある[14]．

ヒトiPS細胞から分化誘導した標的細胞を使って薬剤スクリーニング系を構築する際には，その標的細胞を純度高く，大量に準備することが必要になる．これを実現するためには，目的の細胞を効率よく分化する技術に加え，その細胞を純化する技術が必要になる．iPS細胞間の比較を行う際にも，クローン間での目的細胞への分化効率や分化速度が異なることが知られており，均一な状態で比較検討することが求められる．具体的には目的細胞特異的な表面抗原を認識する抗体で標識をした細胞をフローサイトメトリーで回収する方法などが提案されている[15]．今後，疾患特異的iPS細胞を用いた創薬開発への応用には，更に効率の良い分化誘導・純化法の開発が望まれる．

4. 神経変性疾患モデルとしてのiPS細胞の可能性

これまで，iPS細胞作製技術を利用して疾患再現が行われた疾患は発生過程における異常がその病態に反映される疾患が多い．一方で加齢性の神経変性疾患は，発生過程における異常の存在は不明である．更に，神経細胞に分化誘導し，成熟化した後に初めて何らかの違いが見いだされる可能性がある．加齢変化を*in vitro*で再現するために，長期の培養や更には加齢を促進する培養条件の検討が必要かもしれない．

最近，パーキンソン病の原因遺伝子として知られている，leucine-rich repeat kinase 2（*LRRK2*）遺伝子の変異（G2019S）を有する患者由来のiPS（G2019-iPS）細胞を使った研究が報告された[12]．中脳ドーパミン作動性神経に分化誘導し，コントロール細胞と比較を行ったところ，G2019-iPS細胞において酸化ストレス経路にかかわる*HSPB1*，*NOX1*，*MAOB*といった遺伝子の発現上昇が存在していた．また，*LRRK2*の機能獲得型変異を特徴づけるα-synucleinの蓄積がG2019-iPS細胞において有意に上昇していることが観察された．更に，過酸化水素や6-hydroxydopamine，MG-132の投与によってG2019-iPS細胞から分化誘導したドーパミン作動性神経細胞の神経細胞死が誘導された．この報告では，疾患群，非疾患群の細胞がそれぞれ1クローンであるが，加齢性神経変性疾患モデルとしてのプラットフォームを提示している．

アルツハイマー病では患者の大部分が孤発性であり，遺伝的な原因が不明である．遺伝性の変異を有するアルツハイマー病患者から疾患特異的iPS細胞を作製し，病態を反映する神経細胞へ分化させて神経細胞の変性・細胞死のメカニズムを解明することにより，孤発性患者の治療法，創薬開発につながる知見が得られる可能性がある．

おわりに

本稿では，iPS細胞の疾患モデルとしての現状および展望について述べた．iPS細胞技術という革新的な技術が，今後，アルツハイマー病などの認知症の研究に利用されることで，病態解明，治療法開発が進展することが期待される．

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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.

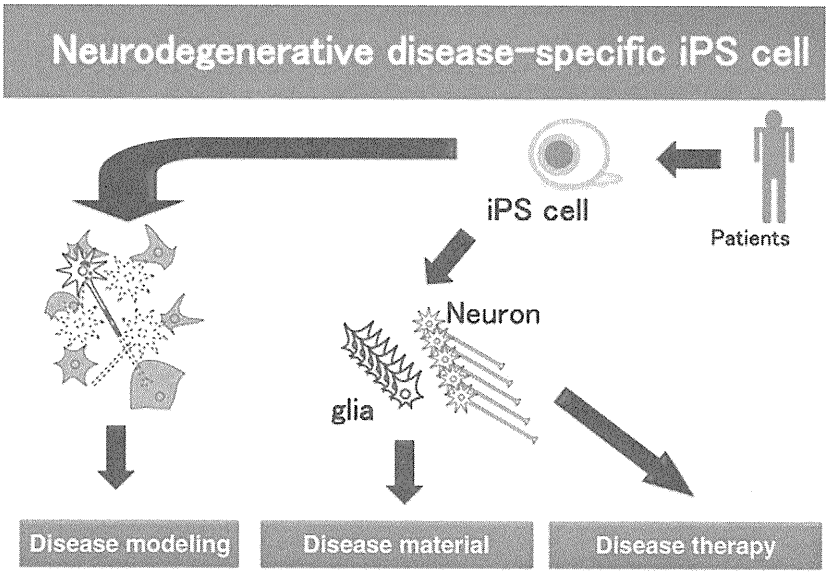

Fig. 1 – Neurodegenerative disease-specific iPSC research contributes to the following 3 areas; "Disease modeling", "Disease material" and "Disease therapy".

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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