標準化）することにより，最終製品の品質を確保しようとする試み（Quality-by-Design）がなされている．これは，自動車・建築・コンピューターなど，設計可能性の高い一般的な工学製品の製造・品質管理方法を医薬品の製造・品質管理に適用しようとするものであるが，原材料の不確実性の高さから考えれば，少なくとも現時点において，細胞・組織加工製品の製造にはQuality-by-Design の思想を適用することは不可能である．

C-4-3 細胞基材の品質管理における留意事項

ICH-Q5D ガイドラインの構成は，①細胞基材（細胞株）の起源．履歴・調製（すなわちドナー情報・培養歴及び株化の方法など），②細胞のバンク化の手法，および③セル・バンクの特性解析となっている．なお，セル・バンクの特性解析としては，特性解析試験，純度試験，細胞基材の安定性，核型分析・造腫瘍性試験が挙げられている．これらをまとめると，バイオロジクス製造用細胞基材の主な留意事項が，「汚染がないことの保証」と，「同一性・均質性の確認・維持」であることが分かる．

例えば，米国 WiCell 研究所 WISC Bank の保有する「臨床グレード」のヒト ES 細胞株（WA09 株）の品質管理においては，1) 主要なウイルス・感染因子のチェックが実施されると同時に 2) フィーダー細胞フリー培養系（TeSR，マトリゲル）を使用し，感染因子の混入を防ぐと同時に，3) 融解後生存率，4) 同一性試験(STR 検査)，5) 染色体異常検査（G バンド検査），および 6) ES 細胞マーカー発現の検査によって，同一性・均質性の確認・維持が行われている．

ここで注意しなければならないのは，WA09 株のような，臨床用途・最終製品が未特定な「臨床グレード」のセル・バンクにおける上記 1)~6) のような留意事項と，特定の細胞・組織加工製品の製造という目的に適ったセル・バンクの品質管理における留意事項とは同じとは限らないということである．

2011 年にハーバード大学の Bock，Kiskinis らは，20 株の ES 細胞と 12 株の iPS 細胞の分化傾向（プロペンシティ）を評価する目的で，各株の細胞を用いて形成させた胚葉体中の細胞種マーカー，胚葉マーカーの発現を検討したデータを報告している（Bock C *et al., Cell.* 2011;144:439-52）．この報告では，各多能性細胞株は確かに多能性を保有するものの，株間で分化プロペンシティのプロファイルが様々であることが示されている．即ち，ヒト ES/iPS 細胞株のセル・バンクを「未分化度」や「多能性」のみで品質管理した場合，目的とする細胞への分化効率にバラツキが生じる恐れがある．従って，多能性幹細胞加工製品の細胞基材としてのセル・バンクにおいては，「目的に適った分化プロペンシティ」を品質特性とする必要性があるかも知れない．2011 年 12 月，動物由来成分を全く使用せずに「臨床グレード」のヒト ES 細胞が樹立され，UK Stem Cell Bank に寄託されたとのニュースが Nature News で報道されたが，同報道には樹立者のコメントとして「実際にヒトに投与されるまでには何年もかかるかもしれない」「細胞株間で組織形成能力は様々であり，心筋を作りやすい株や軟骨を作りやすい株などが存在するので，臨床グレードの株の一連のセットが必要だ」ということが記されている（Callaway E. *Nature News* doi: 10.1038/nature. 2011. 9566）．ヒト多能性幹細胞株／バンクの分化プロペンシティの予測と管理は，今後のヒト多能性幹細胞加工製品の実用化の上で非常に重要な課題となると予想される．

D．考察

ヒト多能性幹細胞加工製品を含む細胞・組織加工製品の製造においては，一定の品質の最終目的製品を安定的かつ継続的に製造する上で重要かつ科学的に合理的な場合に，セル・バンク・システムの構築またはその他の細胞基材の調製が必要となる．基本的には，特定の製品の製造という目的に適った品質の細胞基材としてのセル・バンクは製品の開発者が作成するものだと考えるべきである（図 1）．その際には，最終製品または中間製品の品質・安全性・有効性を基に，適切な親細胞（株）を選択する必要がある．

細胞寄託機関等が供給する「臨床グレード」

のセル・バンクは，最終製品の品質を安定的かつ継続的に確保するために重要かつ科学的に合理的である場合，つまり製品製造という目的に適う場合において利用可能であるが，細胞寄託機関等の「臨床グレード」セル・バンクを利用することが製品製造の必須条件だというわけではない．むしろ，特定の製品を再現性良く製造するためのセル・バンクを，感染因子・免疫原性因子の混入を避けつつ，いかに効率的かつ安価に樹立できるかどうかの方が製品の製造と実用化においては重要と考えられる．

E．結論

細胞基材としてのセル・バンクの品質は，個々の先集製品の品質・態様・適用法・対象疾患等で決まる．細胞株／セル・バンク・システムの「標準化」はデータの相互参照性という意味において学問的には重要であるが，細胞・組織加工製品の製造においては「はじめにセル・バンクの品質（もしくは標準化）ありき」ということはありえず，一定品質の細胞・組織加工製品を再現性良く製造するためにセル・バンクの品質・規格が決定される．標準化された部品・原材料から最終製品の品質が設計可能な多くの工業製品（建築，機械からコンピュータープログラムまで）の製造法と同様な発想を，細胞・組織加工製品の製造に当てはめることは不可能であり合理的ではない．

一般的留意事項のみを満たした「臨床グレード」のセル・バンクから特定の細胞・組織加工製品を製造する場合には，それまで管理されていなかった幾つかのセル・バンクの特性のバラツキにより，目的とする最終製品の品質が確保できない可能性がある．従って，製品ごとに具体的目的に適った品質のセル・バンクが必要となる．細胞寄託機関等が供給する「臨床グレード」のセル・バンクは，安価で簡単にアクセス可能な整理された細胞基材供給源（親細胞株）として有用な可能性がある．ただしその場合でも開発者はそこから改めて特定の製品製造に適う品質のセル・バンクを作成することが必要だと考えられる．

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H. 知的財産権の出願・登録状況

H-1 取得特許 なし

H-2 実用新案登録 なし

H-3 その他 なし

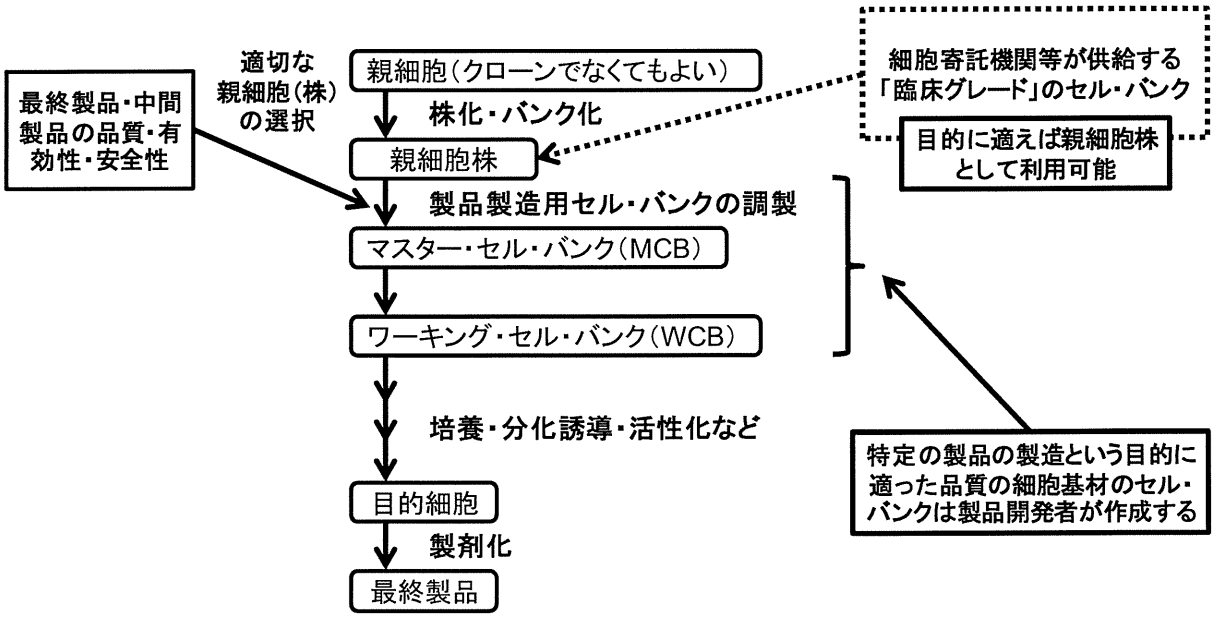

図 1　細胞・組織加工製品の製造の概略

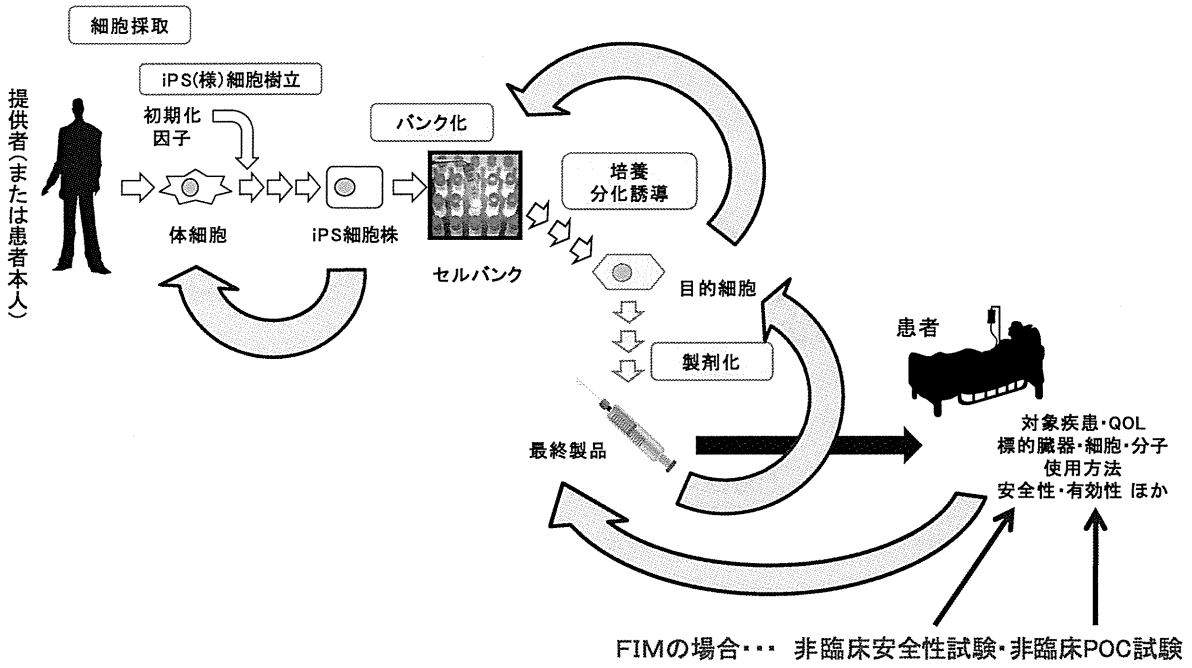

図 2　ヒト iPS 細胞加工製品・原材料の品質は逆行性に規定される

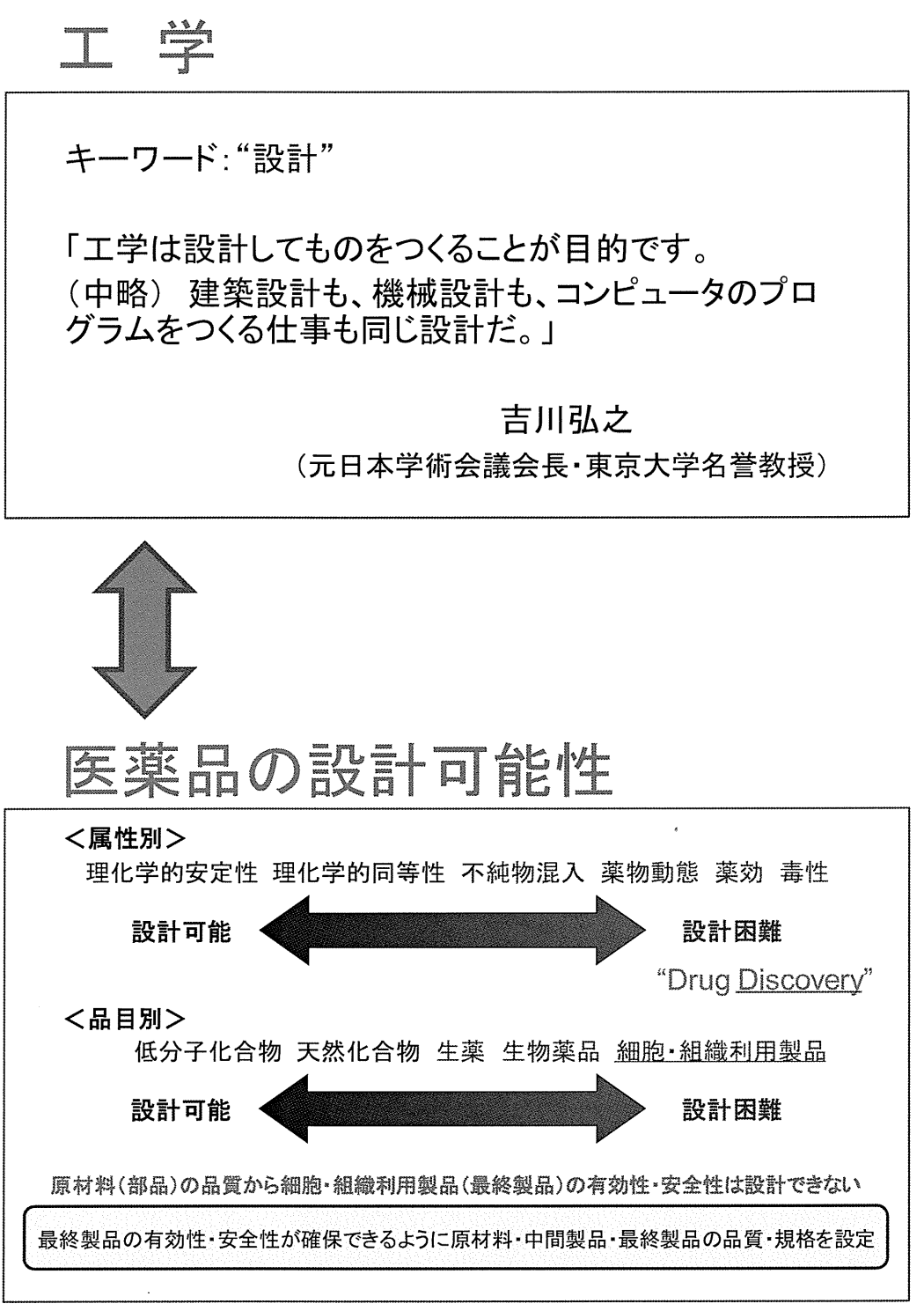

図３　工学における設計の位置づけと医薬品の設計可能性

厚生労働科学研究費補助金（再生医療実用化研究事業）
分担研究報告書

霊長類ES細胞の多能性維持機構，自己複製能に関する基盤研究

研究分担者：末盛 博文　京都大学再生医科学研究所・霊長類胚性幹細胞研究領域 准教授

研究要旨：ＥＳ細胞を用いた細胞移植医療においてその原材料と言えるＥＳ細胞自体の安全性の確保は非常に重要である。様々な合成培養系を検討し、培養液・基質ともに十分な品質管理がなされた条件下での未分化維持培養の可能性を検討した。合成培養系での培養が可能であるが、長期培養時にゲノム安定性に関してより詳細な分析が今後とも必要である。

Ａ．研究目的

ＥＳ細胞などの幹細胞を用いた様々な臨床利用に向けての基盤研究が進展しており、実際に臨床への適用が近づいてくる中で、このような新しい医療を実現する上で、製造管理や品質試験、前臨床試験やその評価方法などについて汎用性の高い技術基盤を構築することは、ＥＳ細胞医療の安全性・有効性を確保する上で非常に重要であるが、いまだ十二分に確立されているとは言えない状況にある。

そこで本研究では、このような再生医療の実用化のため、移植組織の主たる出発材料であるＥＳ細胞の原材料としての安全性をどのように確立するかを、ＥＳ細胞の自己増殖機構の解明とその利用という観点から、培養技術論的なアプローチを中心に検討する。この研究により「安全なＥＳ細胞」を再生医療に供する科学的・技術的基盤の構築に資することを目的とする。

Ｂ．研究方法

「細胞・組織加工医薬品等の品質及び安全性の確認申請記載要領(薬食審査発0420第1号平成22年4月20日)」など関連する指針等に留意し、ＥＳ細胞の臨床利用を実現する上で問題となりうる培養技術に関連した要素について検討する。

培養液・基質などについて高度な品質管理が可能ないわゆる合成培地・基質の性能評価と、これらに使用による細胞の品質に対する影響を分析する。

（倫理面への配慮）

京都大学再生医科学研究所におけるヒトES細胞株の樹立研究と使用研究は、政府指針に沿った文部科学大臣からの確認をすでに受けている。本研究はこれらの研究に含まれる。

Ｃ．研究結果

ヒト多能性幹細胞の臨床応用では、安全性を確保するうえで重要な問題として培養環境から品質管理が困難な動物由来成分などを以下に排除するか、また培養過程で生じる遺伝子変化にどのような生物学的意味があるかが挙げられている。この問題へのアプローチとして本年度は、動物由来成分を含まない培養液、あるいは動物由来成分であっても高度に精製されているなど品質管理可能な培養液と合成基質を用いたヒトＥＳ細胞の培養技術開発を行い、１０継代以上に渡り安定して培養可能であることを見いだした。

また、長期培養時にヒトＥＳ細胞のゲノムに生じる変異の大規模国際共同研究により高頻度変異部位が明らかにされたことから、このような培養条件の変化とゲノム変異の関連性について解析が進むと期待される。

Ｄ．考察

培養液、基質とも開発が進められており実用的なレベルのものが安定的に供給可能な状態に近づいていると言えるだろう。一方で、これらのより管理された環境下でどの程度の期間

安定的に細胞が維持されるのが、ゲノム等に生じる変化の程度・頻度に影響があるのか等については明らかでなく、今後とも慎重に解析を進める必要がある。

E．結論

移植に用いる各種組織の作成に用いられるＥＳ細胞は原材料でありその品質の確保は非常に重要である。ウイルス等の感染性因子に関してはドナーの、あるいは細胞自体の検査によりかなりの程度そのリスクを低減できると見られる。ゲノムの変異に関してはその及ぼす影響をあらかじめ予見することが現時点では困難であり、過剰にリスクを見積もることは患者の治療機会の喪失にもつながるため、バランスの取れた評価を行うことが必要であろう。

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H．知的財産権の出願・登録状況

1．取得特許

なし

2．実用新案登録

なし

3．その他

III. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

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IV. 研究成果の刊行物・別刷

Sugawara *et al. Stem Cell Research & Therapy* 2012, 3:8
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stem cell research&therapy

REVIEW

Investigating cellular identity and manipulating cell fate using induced pluripotent stem cells

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Abstract

Induced pluripotent stem (iPS) cells, obtained from reprogramming somatic cells by ectopic expression of a defined set of transcription factors or chemicals, are expected to be used as differentiated cells for drug screening or evaluations of drug toxicity and cell replacement therapies. As pluripotent stem cells, iPS cells are similar to embryonic stem (ES) cells in morphology and marker expression. Several types of iPS cells have been generated using combinations of reprogramming molecules and/or small chemical compounds from different types of tissues. A comprehensive approach, such as global gene or microRNA expression analysis and whole genomic DNA methylation profiling, has demonstrated that iPS cells are similar to their embryonic counterparts. Considering the substantial variation among iPS cell lines reported to date, the safety and therapeutic implications of these differences should be thoroughly evaluated before they are used in cell therapies. Here, we review recent research defining the concept of standardization for iPS cells, their ability to differentiate and the identity of the differentiated cells.

The potential of stem cells and reprogramming

During mammalian development, cells in the developing fetus gradually become more committed to their specific lineage. The cellular differentiation process specializes to achieve a particular biological function in the adult, and the potential to differentiate is lost. Cellular differentiation has traditionally been thought of as a unidirectional process, during which a totipotent fertilized zygote becomes pluripotent, multipotent, and terminally differentiated, losing phenotypic plasticity (Figure 1). However, recent cloning experiments using nuclear transplantation have demonstrated that the epigenetic constraints imposed upon differentiation in mammalian oocytes can be released and the adult somatic nucleus restored to a totipotent embryonic state [1]. This process, a rewinding of the developmental clock, is termed nuclear reprogramming.

Embryonic stem (ES) cells derived from the inner cell mass of the mammalian blastocyst, an early-stage embryo, were first established from mice by Evans and Kaufman in 1981 [2]. Approximately two decades later, a human ES (hES) cell line was established by Thomson and colleagues [3]. ES cells possess a nearly unlimited capacity for self-renewal and pluripotency: the ability to differentiate into cells of three germ layers. This unique property might be useful to generate a sufficient amount of any differentiated cell type for drug screening or evaluations of drug toxicity and for cell replacement therapy. In addition, pluripotent stem cells provide us with an opportunity to understand early human embryonic development and cellular differentiation. Pluripotent ES cells are spun off directly from pre-implantation embryos [2-5]. To induce the somatic cell back to a pluripotent state, a strategy such as nuclear transplantation is fraught with technical complications and ethical issues. Thus, the direct generation of pluripotent cells without the use of embryonic material has been deemed a more suitable approach that lends itself well to mechanistic analysis and has fewer ethical implications [6].

In a breakthrough experiment, Takahashi and Yamanaka [7] identified reprogramming factors normally expressed in ES cells, Oct3/4, Sox2, c-Myc, and Klf4, that were sufficient to reprogram mouse fibroblasts to become pluripotent stem cells closely resembling ES cells. Because they were induced by the expression of defined factors, these cells were termed induced pluripotent stem (iPS) cells [7]. Since this landmark report in 2006, the technology has been rapidly confirmed among a number of species, including humans [8,9], rhesus monkeys [10], rats [11,12], rabbits [13], pigs [14] and two endangered primates [15]. In addition, mouse iPS (miPS) cells can be derived from various cell types, including fibroblasts [7,16], neural cells [17,18], liver cells [19], pancreatic β

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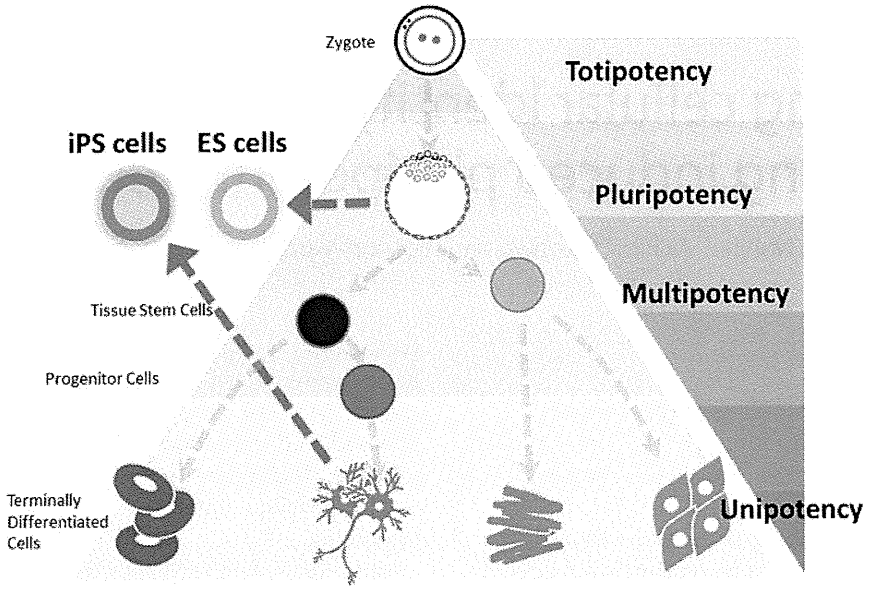

Figure 1. Hierarchical potential of stem cell development. A totipotent cell, such as a zygote and a blastomere of an early pre-implantation embryo, can give rise to all of the cell types in the whole body and the extraembryonic tissues. During mammalian development, pluripotent cells of the inner cell mass differentiate to give rise to lineage-committing stem cells and progenitor cells, and finally terminally differentiated cells by losing differential potential. Embryonic stem (ES) cells are spun off directly from the inner cell mass of blastocysts and induced pluripotent stem (iPS) cells are generated by reprogramming differentiated cells back to the pluripotent state. ES cells and iPS cells seem to have highly similar pluripotential properties.

cells [20], and terminally differentiated lymphocytes [21,22]. Subsequently, human iPS (hiPS) cells have been derived from various readily accessible cell types, including skin fibroblasts [8,9], keratinocytes [23], gingival fibroblasts [24], peripheral blood cells [25,26], cord blood cells [27,28] and hair follicle cells [29].

These products and systems for this state-of-the art technology provide useful platforms for disease modeling and drug discovery, and could enable autologous cell transplantation in the future. Given the methodologies for studying disease mechanisms, disease- and patient-specific iPS cells can be derived from patients. For applying novel reprogramming technologies to biomedical fields, we need to determine the essential features of iPS cells. In this review, we summarize the functional and molecular properties of iPS cells in comparison to ES cells in the undifferentiated state and with regard to differentiation efficiency. We also review evaluation for the types of differentiated cells derived from of iPS and ES cells and compare the functions of these.

Reprogramming methods and factors

Although the establishment of iPS cells from somatic cells is technically easier and simpler compared with nuclear transplantation, several variables should be considered due to variations in the reprogramming process, including the reprogramming factors used, the combinations of factors and the types of donor-parent cells. Each method has advantages and disadvantages, such as efficiency of reprogramming, safety, and complexity, with the process used affecting the quality of the resultant iPS cells. Initial generations of miPS and hiPS cells employed retroviral and lentiviral vectors [7-9] (Table 1), carrying the risk of both insertional mutagenesis and oncogenesis due to misexpression of the exogenous reprogramming factors, Oct3/4, Sox2, c-Myc, and Klf4. In particular, reactivation of c-Myc increases tumorigenicity in the chimeras and progeny mice, hindering clinical applications.

Since the initial report of iPS cell generation, modifications to the reprogramming process have been made in order to decrease the risk of tumorigenicity and increase reprogramming efficiency [30-32]. Several small molecules and additional factors have been reported to enhance the reprogramming process and/or functionally replace the role of some of the transcription factors (Table 1). Small molecules are easy to use and do not result in permanent genome modifications, although iPS generation using only a set of small molecules has not been reported. Combining small molecule compounds with reprogramming factors would enhance reprogramming efficiency. Integration-free hiPS cells have been established using Sendai virus [33,34], episomal plasmid vectors [35,36], minicircle vectors [37], and direct protein

Table 1. Various methods used for reprogramming

Method	Factors[a]	Sources	Enhancement factors
Adenovirus	OSKM	Mouse fibroblast and liver cells [77], human embryonic fibroblast cells [78]	
Bacteriophage	OSKM	Mouse embryonic fibroblasts, human amniocytes [79]	
Episomal vector	OSKMNL	Human foreskin fibroblasts [36]	SV40LT
		Human fibroblasts, adipose stem cells, cod blood cells [80]	SV40LT, LIF, MEK/GSK3b/TGFBR inhibitor, HA-100/human
	OSKM*L	Human dermal fibroblasts [81]	p53 shRNA
Lentivirus	OSKM	Mouse pancreatic b cells [20]	
		Human adult fibroblasts [82]	p53 siRNA, UTF1
		Mouse B lymphocytes [21]	C/EBPa or Pax5 shRNA
	OSNL	Human newborn foreskin [9]	
		Human fibroblasts [83]	SV40LT
	OSKMNL	Human fibroblasts [84]	
	OSN	Gut mesentery-derived cells [85], human amnion-derived cells [86]	
	O	Human epidermal keratinocytes [87]	TGFBR/MEK1 inhibitor, PDK1 activator, sodium butyrate
Minicircle vector	OSNL	Human adipose stromal cells [37]	
microRNA	miR-200c, 302a/b/c/d, 369-3p/5p	Human and mouse adipose stromal cells [64]	
mRNA	OSNL	Human fibroblasts [88]	
	OSKM(L)	Primary human neonatal epidermal keratinocytes [40]	
piggyBAC	OSKM	Human and mouse embryonic fibroblasts [89,90]	
Plasmid	OSKM	Mouse embryonic fibroblasts [35,91]	
	OSNL	Human foreskin fibroblasts [92]	MEK inhibitor
Protein	OSKM	Mouse embryonic fibroblasts [38]	VPA
	OSKM	Human fibroblasts [39]	
Retrovirus	OSKM	Human fibroblasts [8], mouse fibroblasts [7], human keratinocytes [23], human peripheral blood cells [25]	
		Human fibroblasts, adipose stem cells [93]	Vitamin C, VPA
	OSK	Adult human dermal fibroblasts [30]	
		Mouse embryonic fibroblasts [94]	Wnt3a
		Rat liver progenitor cells [11]	MEK/ALK5/GSK3b inhibitor
		Mouse embryonic fibroblasts [93]	Vitamin C
		Mouse and human fibroblasts [32]	GLIS1
		Mouse embryonic fibroblasts [95]	mmu-miR-106a/18b/20b/19b/92a/363 or 302a/302b/302c/302d/367
		Human fibroblasts [96]	hsa-miR-302b or 372
	OK	Mouse embryonic fibroblasts [97]	BIX01294, BayK8644
		Neonatal human epidermal keratinocytes [98]	GSK3b inhibitor
	O	Mouse neural stem cells [99]	
		Mouse fibroblasts [100]	GSK3b inhibitor, vitamin C, BMP4
	hsa-miR-302a/b/c/d	Human skin cancer cells [101]	
Sendai virus	OSKM	Human fibroblasts [33], human cord blood [102]	

[a]O, OCT3/4; S, SOX2; K, KLF4; M, C-MYC; M*, L-MYC; N, NANOG; L, LIN28. ALK, anaplastic lymphoma kinase; BayK8644, L-type calcium channel agonist; BIX01294, histone methyltransferase inhibitor; BMP, bone morphogenetic protein; GSK, glycogen synthase kinase; GLIS, GLI (MIM 165220)-related Kruppel-like zinc finger; LIF, leukemia inhibitory factor; PDK, pyruvate dehydrogenase kinase; shRNA, short hairpin RNA; siRNA, small interfering RNA; TGFBR, transforming growth factor beta receptor; UTF, undifferentiated transcription factor; VPA, valproic acid (histone deacetylase inhibitor).

[38,39] or mRNA [40] delivery (Table 1). However, direct delivery of proteins or RNA requires multiple transfection steps with reprogramming factors compared to other viral integration methods.

iPS cells appear indistinguishable from ES cells

The key to generating iPS cells is to revert somatic cells to a pluripotent state that is molecularly and functionally equivalent to ES cells derived from blastocysts (Table 2). Reprogrammed iPS cells express endogenous transcription factors that are required for self-renewal and maintenance of pluripotency, such as OCT3/4, SOX2, and NANOG, and for unlimited proliferation potential, such as TERT [8,9]. Telomeres were elongated in iPS cells compared to the parental differentiated cells in both humans and mice [41,42]. In addition, cellular organelles such as mitochondria within hiPS cells were morphologically and functionally similar to those within ES cells [43]. The establishment of an ES cell-like epigenetic state is a critical step during the reprogramming of somatic cells to iPS cells and occurs through activation of endogenous pluripotency related genes. Bisulfite genomic sequencing has shown that the promoter regions of the pluripotency markers NANOG and OCT3/4 are significantly demethylated in both hiPS and hES cells [8,44], and the heterogeneity of X chromosome inactivation in hiPS cells is similar to that in ES cells [45].

In terms of multilineage differentiation capacity, miPS cells from various tissue types have been shown to be competent for germline chimeras [19,32,46]. It was shown that miPS cells generated viable mice via tetraploid complementation [47,48]. In the mouse system, iPS cells retain a developmental pluripotency highly similar to that of mouse ES cells according to the most stringent tests. Although it has been generally assumed that autologous cells should be immune-tolerated by the recipient from whom the iPS cells were derived, Zhao and colleagues [49] reported that the transplantation of immature miPS cells induced a T-cell-dependent immune response even in a syngeneic mouse. This is an unexpected result but some issues need to be considered: the influence of the cell type of origin on the immunogenic properties of resultant iPS cells must be explored; undifferentiated iPSCs should never be used for medical applications; and the mechanism of aberrant gene expression should be determined [50].

To functionally assay hiPS cells, teratoma formation and histological analysis to confirm the presence of structures derived from all three germ layers are currently regarded as the most rigorous ways to prove pluripotency of human stem cells. Recently, Müller and colleagues [51] proposed the use of PluriTest, a bioinformatics assay for the prediction of stem cell pluripotency using microarray data. Such microarray-based gene expression and DNA methylation assays are low cost, save time and have been used to evaluate the differentiation efficiency of individual cell lines [52].

ES and iPS cells differ in their epigenetic signatures

Epigenetic modification of the genome ensures proper gene activation for maintaining the pluripotency of stem cells and also differentiation into proper functional cells [1]. It will be important to assess the epigenetic state of hiPS cells compared to donor parent cells and embryo-derived hES cells. Analyzing epigenetic states, such as histone modifications and DNA methylation of selected key pluripotency genes, showed the chromatin state of iPS cells to be identical to that of ES cells upon reprogramming (reviewed in [53]).

Genome-wide analyses of histone methylation patterns have demonstrated that iPS cells were clearly distinguished from their origin and similar to ES cells in the mouse [54]. All of these analyses, however, reported some differentially methylated regions (DMRs) between ES and iPS cells. Recent studies found that miPS cell lines retained the residual signatures of DNA methylation of the parental cells [55,56]. Additionally, some of the hyper-methylated regions in hiPS cells are also hyper-methylated in the original cells, meaning that an epigenetic memory is inherited during the reprogramming process through early passaging [57]. Parental cell-related DMRs and incomplete promoter DNA methylation contributed to aberrant gene expression profiles in iPS cells to some extent [58]. The other remaining DMRs appeared to be aberrantly methylated regions established in iPS cells during reprogramming that differ from both the parental cells and the ES cells. Nishino and colleagues [57] compared methylation profiles of six hiPS cell lines and two hES cell lines and reported that approximately 60% of DMRs were inherited and 40% were iPS-specific. Interestingly, most aberrant DMRs were hyper-methylated in iPS cell lines [57,59]. Lister and colleagues [60] also compared methylation profiles in five hiPS cell lines and two hES cell lines and found that the hiPS cells shared megabase-scale DMRs proximal to centromeres and telomeres that display incomplete reprogramming of non-CpG methylation, and differences in CpG methylation and histone modifications in over a thousand DMRs between hES and hiPS cells. Although lots of studies have detected several DMRs shared between iPS and ES cells, no DMRs were found in all iPS cell lines.

microRNAs (miRNAs), which are also epigenetically regulated, play critical roles in gene regulation by targeting specific mRNAs for degradation or by suppressing their translation. Several studies recently reported the presence of unique clusters of miRNAs, such as the human and mouse miR-302 cluster in ES and iPS cells [61,62]. These miRNAs enhance the transcription factor-mediated

Table 2. Characteristics of human induced pluripotent stem cells compared to human embryonic stem cells

Variable factor	Characteristics	Characteristics of hiPS cells
Cell source		Without the use of embryonic material Enable autologous cell transplantation
Technique for the generation of iPS cells		Simply trans-activating several transcription factors and/or exposure to several chemical components Variables due to reprogramming methods and/or donor-parental cells
Morphology		Flat and tightly packed colony identical to hES cells
Proliferation potency		Unlimited self-renewal identical to hES cells
Pluripotency	Genes	OCT3/4, NANOG, SOX2 expression identical to hES cells
	Gene promoter	OCT3/4, NANOG demethylation identical to hES cells
	Cell surface antigens	SSEA3, SSEA4, TRA-1-60, TRA-1-81 positive identical to hES cells
	Teratoma formation	Differentiation into three germ layers similar to hES cells
X chromosome inactivation (XCI)		Heterogeneity (complete XCI, partial XCI, pre-XCI) similar to hES cells
Mitochondria	Genome	Accumulated mtDNA mutations transmitted from parental cells Genetic mutations during reprogramming
	Morphology	Globular shape with only small christae similar to hES cells and ES cell-like distribution
	Function	Expression of nuclear factors involved in mitochondrial biogenesis
Telomere		Telomere elongation and ES cell-like telomerase activity
Epigenetic profile		Retention of somatic memory and aberrant methylation during the reprogramming process
microRNAs		Up-regulation of miR-302 cluster identical to hES cells

ES, embryonic stem; hES, human embryonic stem; hiPS, human induced pluripotent stem; iPS, induced pluripotent stem; mtDNA, mitochondrial DNA; XCI, X chromosome inactivation.

reprogramming process (Table 1). Furthermore, two independent groups generated human and mouse iPS cells by adding only miRNAs in the absence of any additional protein factors [63,64]. Two reports have described a small number of differences in miRNA expression patterns between hiPS and hES cells [62,65], although our preliminary analysis showed that miR-372 and miR-373 are expressed at similar levels in both hiPS and hES cells and they were not detected in parental cells.

Changes of epigenetic profiles in iPS cells during culture

It is possible that iPS cells vary in their epigenetic profiles and degree of pluripotency due to differential levels of reprogramming. Nishino and colleagues [66] investigated the effect of continuous passaging on DNA methylation profiles of seven hiPS cell lines derived from five cell types. Although *de novo* DMRs that differ between hES and hiPS cells appeared at each passage, their number decreased and they disappeared with passaging; therefore, the total number of DMRs that differ between ES and iPS cells decreased with passaging. Thus, continuous passaging of the iPS cells diminished the epigenetic differences between iPS and ES cells, implying that iPS cells lose the characteristics inherited from the parental cells and develop to very closely resemble ES cells over time [66]. They also confirmed that the transgenes were silenced at each passage examined, indicating that the number of DMRs that differed between ES and iPS cells decreased during the transgene-independent phase. This is consistent with a study by Chin and colleagues [67], who found that the gene expression profile of hiPS cells appeared to become more similar to that of hES cells upon extended passaging. Although comprehensive DNA methylation profiles have recently been generated for hiPS cells, it seems harder to determine common DMR sites during iPS reprogramming. There are three possible explanations for the many inconsistent results regarding iPS cell-specific DMRs: hiPS cells have only been analyzed at a single point of passage in almost all studies; inherited methylation from parental cells is non-synchronous and stochastic, much like aberrant methylation, rather than deterministic [66]; and the aberrant hypermethylation at DMRs in iPS cells occurs 'stochastically' throughout the genome during passaging [66].

Genetic changes during reprogramming and extended culture

Genomic stability is critical for the clinical use of hiPS cells. The occurrence of genetic changes in hES cells is now well known as well as that the karyotypic changes observed are nonrandom and commonly affect only a few chromosomes [68]. Recent studies revealed that the