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—Review—
Review Series: Animal Bioresource in Japan

Bio-Resource of Human and Animal-Derived Cell Materials

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Abstract: The Cell Engineering Division of RIKEN BioResource Center is a not-for-profit public "cell bank" that accepts donations and deposits of human and animal cell materials developed by the life science research community. We examine, standardize, amplify, preserve, and provide cell materials to scientists around the world. The major cell materials used around the world have been cultured cell lines, i.e., immortalized cells. Most human cell lines are derived from tumor cells. There is no doubt that the demand for these cell lines will never cease in the field of biology. In addition, stem cell lines such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are of great value in current biology and medical science. Thus, we are extensively collecting such stem cell lines, aiming at contributing to the fields of developmental biology and transplantation/regenerative medicine. In addition, the demand for primary cells has recently increased. To meet this demand, we have started the banking of primary human cells including somatic stem cells, such as umbilical cord blood cells and cultured mesenchymal cells. The staff of the Cell Engineering Division conduct not only the banking of cell materials, but also research and development relating to cell materials, such as the establishment of novel human and animal-derived cell lines and the development of new technology to utilize cell materials.
Key words: cell bank, cell line, ES cells, iPS cells, stem cell bank

Introduction

RIKEN Cell Bank was established as a not-for-profit public cell bank in 1987 when a committee of scientists in Japan recognized the needs of scientists for a central collection of human and animal cell materials. In 2001, RIKEN BioResource Center (RIKEN BRC) was established, and the RIKEN Cell Bank was reorganized into the Cell Engineering Division of RIKEN BRC. In 2002, the Cell Engineering Division of RIKEN BRC was recognized as the central archive for the collection of "human and animal cell materials" in the National BioResource Project (NBRP) program, sponsored by the Ministry of Education, Culture, Sports, Science and Technology.

Cell Materials Available from RIKEN BRC

We possess more than two thousand immortalized cell lines (Table 1), of which approximately 1,500 cell lines

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Table 1. Cell materials available from RIKEN BRC

(1) Mammals	Bovine
	Cat
	Chimpanzee
	Common Marmoset
	Dog
	Elephant
	Hamster
	Human (see Table 2)
	Mink
	Monkey
	Mouse
	Pig
	Rabbit
	Rat
	Suncus
	Tupaia
(2) Birds	Avian
(3) Amphibians	Frog
	Newt
	Salamander
(4) Fishes	Mudminnow
	Eel
	Gold fish
	Medaka
	Tilapia
	Zebrafish
(5) Insects	Drosophila
	Armyworm
	Butterfly
	Moth
	Silkworm
(6) Others	Hybridoma

Table 2. Human cells available from RIKEN BRC

- (1) General cells
  - (1-1) Cancer cell lines
  - (1-2) Primary cells such as fibroblasts
- (2) Cells for genome research
  - (2-1) Healthy people
    - (2-1-1) Japanese
    - (2-1-2) Sonoda-Tajima collection: various racial and ethnic backgrounds
  - (2-2) Patients
    - (2-2-1) Breast cancer
    - (2-2-2) Goto collection: Werner syndrome
- (3) Stem cells
  - (3-1) Somatic stem cells
    - (3-1-1) Umbilical cord blood cells
      - (3-1-1-1) Nuclear cells
      - (3-1-1-2) Mononuclear cells
      - (3-1-1-3) CD34-positive cells
    - (3-1-2) Mesenchymal cells
      - (3-1-2-1) Primary mesenchymal stem cells
      - (3-1-2-2) Immortalized mesenchymal cell lines
  - (3-2) Embryonic stem (ES) cells
  - (3-3) Induced pluripotent stem (iPS) cells
    - (3-3-1) iPS cell lines derived from normal cells
    - (3-3-2) Disease-specific iPS cells (not available yet)

are immediately available for distribution. Approximately half of the cell lines were derived from humans (Table 2) and the other half of the cell lines were derived from various animals (Table 1).

Cell Materials Derived from Various Animals

We provide not only rodent cells but also cells derived from many other kinds of mammals (Table 1). In addition to mammalian cells, we also provide other vertebrate cells such as bird-, amphibian-, and fish-derived cells. In relation to non-vertebrate cells, we provide insect cells as well. Many kinds of hybridoma cell lines, each of which produces a specific monoclonal antibody against a certain antigen, are also available.

Mouse Embryonic Stem (ES) Cell Lines

In addition to many kinds of mouse cell lines of somatic cell origin, we provide many kinds of mouse embryonic stem (ES) cell lines. Not only ES cell lines derived from the 129 strain but also ES cell lines derived from C57BL/6 strain are also available. In particular, two cell lines, B6G-2 (AES0003) [13] and BRC6 (AES0010) derived from C57BL/6N strain, have been confirmed to be differentiable into germ line cells. Thus, both B6G-2 and BRC6 can be used for the establishment of gene knock-out mouse strains with a C57BL/6N background.

We also provide many kinds of nuclear-transferred mouse ES cell lines [18]. Nuclear-transferred ES cell lines are established by reprogramming of somatic nuclei following their transfer into enucleated oocytes. Comparison of conventional ES cell lines with nuclear-transferred ES cell lines is potentially useful for studying the mechanism of reprogramming, and such comparisons may also contribute to understanding the mechanism underlying the establishment of induced pluripotent stem (iPS) cells.

Please refer to the following website for more information on animal ES cell lines.
http://www.brc.riken.jp/lab/cell/english/index_aes.shtml

Common Marmoset ES Cell Lines

We provide an ES cell line derived from the common marmoset, CMES40 (AES0053) [11]. It has recently been reported that a transgenic common marmoset was successfully established [12]. Hence, common marmoset ES cell lines will be very useful in the field of translational research, since it is likely that gene-modified common marmosets will be established using ES cell lines.

Cell Materials for Human Genome Research

To analyze the causes of certain specific diseases at the genomic level, many genome samples are required. However, it is not so easy to collect many samples at a time. Thus, the collection of many genome samples or cell lines containing the genome is very important and useful for researchers in the field.

We have collected two hundred Epstein-Barr virus (EBV)-transformed B cell lines derived from healthy Japanese people. Leukocyte antigen (HLA) haplotypes have been determined for half of them.

We are also collecting EBV-transformed B cell lines derived from cancer patients. At the moment, we possess 48 EBV-transformed B cell lines derived from breast cancer patients in Japan.

Goto Collection

This is a collection of cells derived from patients suffering from Werner syndrome [2]. Werner syndrome is characterized by the premature appearance of features associated with normal aging and cancer predisposition. Compared to progeria syndrome, another premature disease, Werner syndrome is characterized by late onset of symptoms. Of note, the majority of Werner syndrome patients in the world are Japanese. Thus, many scientists around the world are focusing on this collection.

Sonoda-Tajima Collection

Ancestors of Amerindians migrated from the Eurasian continent to North America continent via the Bering Strait, and then migrated to South America continent over 10 thousand years ago. Although human geneticists have tried to find their origin in Eurasia and to trace their migration paths with genetic methods, it has been difficult to obtain sufficient numbers of tissues or cell samples.

Dr. Sonoda who was a professor at Kagoshima University in Japan and Dr. Tajima, who is currently the director of Aichi Cancer Center Institute in Japan, spent nearly 30 years collecting peripheral blood samples from more than 3,500 individuals of Mongoloid minority groups around the world, mainly individuals living in South America (Fig. 1) [5]. Their cell collection was donated to the RIKEN BRC.

We are establishing EBV-transformed B cell lines from the donated peripheral blood samples. We have recently started a service to provide the EBV-transformed B cell lines together with information such as age, gender, tribe, and locality of the originating individual, and also relationships between specimen individuals.

Human Somatic Stem Cells

Compared with primary cells derived from experimental animals, human primary cells are very difficult to obtain. Current research in life sciences, however, requires human primary cells, such as stem cells, particularly in the fields of transplantation and regenerative medicine. We have succeeded in establishing systems for providing such human primary cells efficiently.

Umbilical cord blood is a source of hematopoietic stem cells as well as of other somatic stem cells. Human umbilical cord blood cells are readily available, but are usually discarded if they are not used in transplantation. Provided that the mother of a newborn baby agrees to allow the use of cord blood cells for research purposes, the material can be a valuable resource without the complicating factor of ethical concerns. By collaborating with the "Japanese Cord Blood Bank Network", we are supplying umbilical cord blood cells to domestic researchers in order to contribute to the fields of transplantation and regenerative medicine.

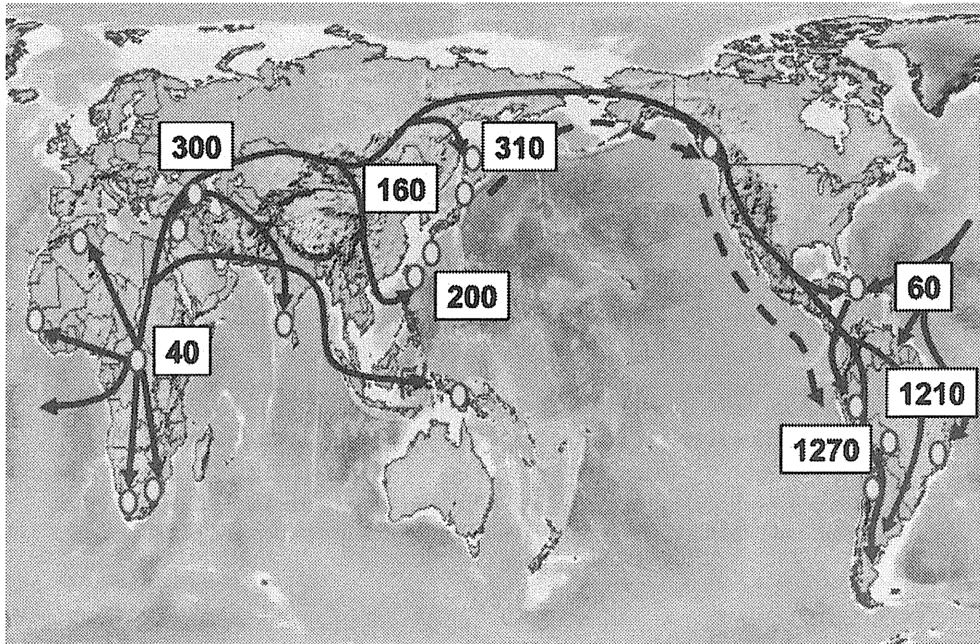

Fig. 1. Sonoda-Tajima collection. This collection contains a great number of cells derived from many people from various areas around the world. The numbers in the figure indicate the sample numbers collected in each area.

By collaborating with researchers in Japan who have developed technologies for expanding human mesenchymal stem cells *in vitro* very efficiently [4, 17], we are supplying human mesenchymal stem cells to domestic researchers. Mesenchymal stem cells can differentiate to bone, cartilage, muscle, tendon, cardiomyocytes, and so on. Thus, mesenchymal stem cells are very attractive and promising materials in the field of regenerative medicine.

Human ES Cells

In April 2008, the Ministry of Education, Culture, Sports, Science and Technology of Japan approved the collection and distribution of human ES cell lines by RIKEN BRC. Human ES cell lines are very useful cell materials in many fields of biology such as developmental biology, regenerative medicine, and drug discovery. We have accepted the deposit of three human ES cell lines established at Kyoto University (KhES-1, KhES-2, KhES-3) [15]. From March 2009 we have been distributing KhES-1 and we anticipate distributing the other two human ES cell lines in the near future.

Induced Pluripotent Stem Cells

Dr. Yamanaka of Kyoto University (Japan) has developed a breakthrough technique in the field of biology. He has enabled the induction of pluripotent stem cells (iPS cells) from somatic cells by using four defined factors. We have accepted the deposit of several iPS cell lines from Dr. Yamanaka: a mouse cell line established with four factors (Oct3/4, Sox2, Klf4, and c-myc in retrovirus vector) (Fig. 2) [8], a mouse cell line established with three factors (Oct3/4, Sox2, and Klf4 in retrovirus vector) [7], two mouse cell lines established with four factors (Oct3/4, Sox2, Klf4, and c-myc in plasmid vector) [9], a human cell line established with four factors (Oct3/4, Sox2, Klf4, and c-myc in retrovirus vector) [16], and a human cell line established with three factors (Oct3/4, Sox2, and Klf4 in retrovirus vector) [7]. We are currently providing all these iPS cell lines.

In the near future, the total number of iPS cell lines derived from patients (disease-specific iPS cells) will tremendously increase [10]. Accordingly, we plan to add them to our collection and provide such disease-specific iPS cell lines as well.

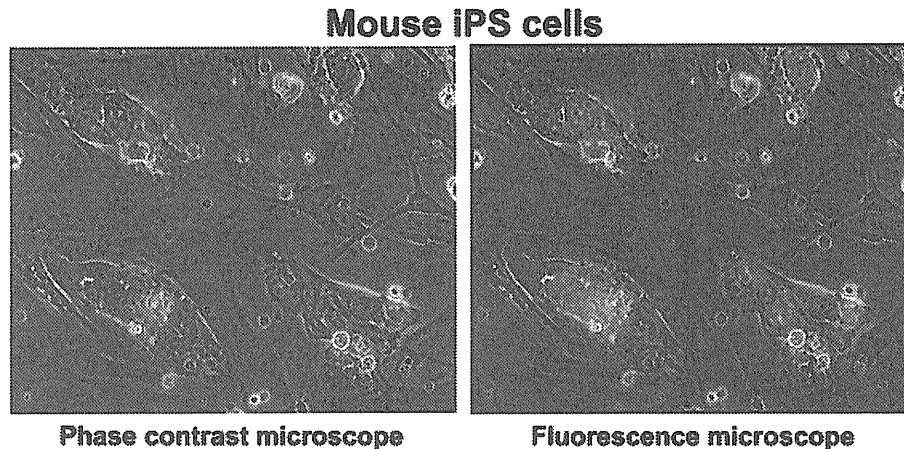

Fig. 2. Mouse iPS cells deposited by Dr. Yamanaka. GFP gene is knocked-in under Nanog promoter, and thus, the expression of GFP can be detected in undifferentiated cells.

Quality Control of Cell Lines

Misidentification of cell lines can result in the generation of erroneous scientific data [1, 14]. Hence, it is very important to eliminate cell lines whose origins differ from those claimed. Interspecies contamination can be detected by various established methods, such as karyotype and isozyme analyses. However, it has been impossible to detect intra-species cross-contamination in the absence of a technology for detecting differences between cell lines at the molecular level. Recently, the profiling of short tandem repeat (STR) polymorphisms (STR profiling) has been established as a method of analyzing gene polymorphism [6, 19]. STR profiling is a simple and reliable method of identifying individual human cell lines. All human cell lines that are currently distributed from our division have been analyzed by STR profiling to authenticate their identity. We found through such analysis that nearly 10% of the deposited cell lines had been misidentified. STR profiling is a useful and powerful method of eliminating cell lines that have been misidentified as a result of cross-contamination or other causes, and STR profiling of human cell lines is routinely performed in major cell banks around the world. If researchers would use only the cell lines that have been tested by STR profiling in cell banks, misidentification of human cell lines would be eradicated.

In relation to the cell lines derived from mice, we are performing simple sequence length polymorphism (SSLP) analysis, an analysis quite similar to STR profiling on human cell lines, to confirm mouse strain origins. Similar to human cell lines, nearly 10% of the deposited mouse cell lines were found to have been misidentified by SSLP analysis.

We have established a quality management system (QMS), and we are continuously performing all works in our laboratory according to this QMS. In July 2007 our QMS was accredited by ISO 9001, and the accreditation has been maintained (Fig. 3).

Ethical Matters Relating to Human Cell Materials

The cell banking of human cells requires strict observation of ethical codes. We only accept human cell donations that are approved by the Institutional Review Board (IRB) at RIKEN Tsukuba Institute. Furthermore, RIKEN BRC contracts a Material Transfer Agreement (MTA) with the organization that deposits or donates human cells at RIKEN BRC. In the MTA, RIKEN BRC receives an assurance from the depositor that the human cell resources were obtained with appropriate informed consent. An approval by the IRB of the organization that deposits or donates human cells to RIKEN BRC is also necessary. When RIKEN BRC distributes human cells to users, we always contract a MTA with them. As

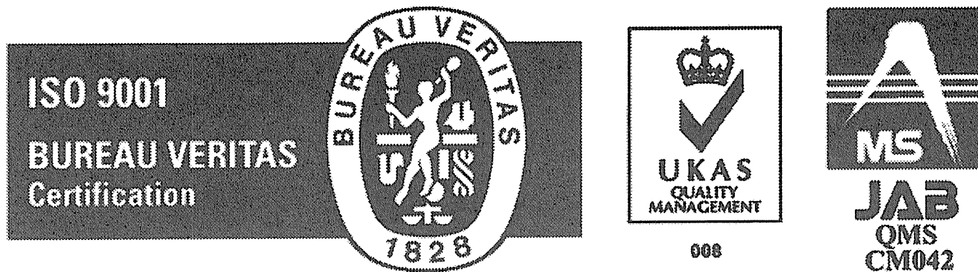

Fig. 3. Accreditation by ISO 9001. All works in RIKEN Cell Bank are performed according to a quality management system (QMS). The QMS has been accredited by ISO 9001.

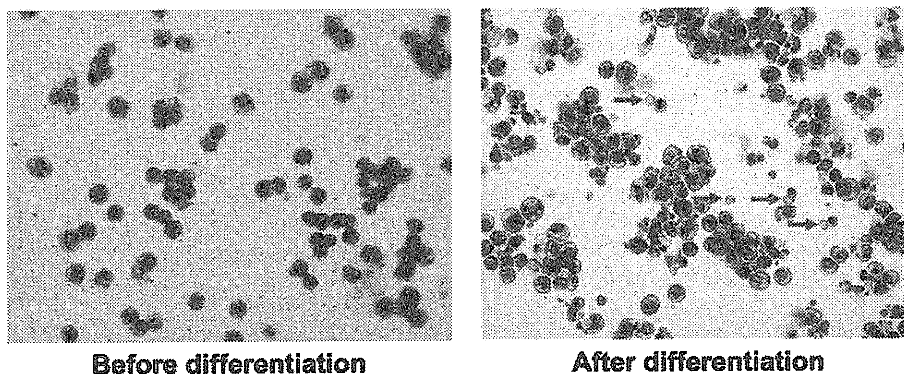

Fig. 4. An erythroid cell line established from mouse ES cells (E14), MEDEP-E14. After induction of differentiation, enucleated red blood cells can be detected. Red arrows indicate enucleated red blood cells.

for certain human cells, such as umbilical cord blood and mesenchymal stem cells, approval by the IRB of the user organization is also required.

Research and Development

Human and animal cell lines with multipotency or tissue-specific features are very useful for developmental biology and basic research in regenerative medicine. We are trying to establish such cell lines by various approaches. First, the identification and purification of tissue-specific somatic stem cells may lead to the establishment of such cell lines by immortalizing such somatic stem cells. Second, the induction of the differentiation of ES or iPS cells may lead to the establishment of such cell lines. In fact, we have recently succeeded in establishing erythroid cell lines from mouse ES cells (Fig. 4) [3]. We are investigating these possibilities using both human and animal cell materials.

All kinds of cells are affected by many factors, i.e., extracellular and intracellular factors, both *in vivo* and *in vitro*. Analyses of the functions of these factors are essential for improving cell culture and cell manipulation. The search for novel factors is also one of the most important research interests in this field.

General Information of RIKEN Cell Bank

In recent years, more than four thousand ampoules have been distributed annually, mostly to not-for-profit organizations (80%) and approximately 10% overseas. We will continue to accept deposits and donations of cultured human and animal cell lines and expand the collection, since the significance of the cell lines in the

field of biology will never cease.

Japanese website of our division is as follows.

http://www.brc.riken.jp/lab/cell/

English website of our division is as follows.

http://www.brc.riken.jp/lab/cell/english/

E-mail address for questions regarding materials and methods is as follows.

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Review Article

Induced pluripotent stem (iPS) cells offer a powerful new tool for the life sciences

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Abstract

Stem cell biology started with the analysis of somatic stem cells that function to maintain the adult body. We now know that the body is maintained by regeneration of a wide range of cell types, such as skin cells, blood cells and gastrointestinal mucous cells, from somatic stem cells. This regenerative activity is essential for survival. Regenerative medicine was initiated to identify therapies that support and/or accelerate this natural regenerative ability. For example, bone marrow transplantation is a therapy for reconstituting hematopoiesis from the hematopoietic stem cells present in the donor bone marrow. The successful development of a protocol for obtaining human embryonic stem (ES) cells prompted medical scientists to utilize human ES cells for regenerative medicine. However, use of these cells raises ethical issues as they are derived from human embryos. An alternative approach using ES-like pluripotent stem cells has the considerable advantage that it does not necessitate use of human embryos. Pluripotent stem cells can be induced from terminally differentiated somatic cells by the introduction of only four defined factors. The products of this method are termed "induced pluripotent stem (iPS)" cells. iPS cells have considerable promise as a substitute for ES cells not only for regenerative medicine but also in many other fields. For example, liver and heart cells derived from iPS cells can be used in pharmaceutical research. In addition, iPS cell technology opens new avenues of disease research, for example, by construction of so-called "disease-specific iPS cells" from a patient's somatic cells.

Key words; ES cells, iPS cells, Regenerative medicine, Disease specific iPS cells

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Prologue

The development of technologies such as PCR and gene knock-out that enable the manipulation of an organism's genetic material contributed tremendously to progress in the life sciences in the final decades of the last century. This century looks to continue this progress through the development of further new technologies such as that described here, the production and manipulation of induced pluripotent stem (iPS) cells.

Discovery of plasticity in terminally differentiated cells

It was believed for a long time that epigenetic modifications in differentiated somatic cells were irreversible. This meant that terminally differentiated cells could never return to being immature cells. However, in 1962 it was reported that the nuclei of somatic cells of an amphibian (frog) were reprogrammed following transfer into enucleated unfertilized eggs[1]. Following transfer of a somatic cell nucleus, the egg could undergo cell division and differentiate to produce an adult frog. This result clearly indicated that epigenetic modifications in terminally differentiated somatic cells were reversible. Dr. John Gurdon, who performed this groundbreaking study, received the Albert Lasker Basic Medical Research Award in 2009.

Initially, many biologists believed that this reversibility of epigenetic modifications in terminally differentiated cells was restricted to amphibian somatic cells and did not occur in mammalian somatic cells. However, in 1997, a nuclear transfer experiment in sheep in which somatic nuclei were transferred into unfertilized eggs showed that epigenetic modifications in terminally differentiated mammalian somatic cells were also reversible[2]. This experiment famously resulted in the birth of the first live cloned sheep, named "Dolly".

Immortalization of embryonic stem cells

The methodology for isolating and culturing mouse embryonic stem (ES) cells was first developed in 1981[3] and has aided research in a wide range of biological studies. Dr. Martin Evans, who developed the technology for establishing mouse ES cell lines, was awarded a Nobel Prize in 2007 together with Dr. Mario Capecchi and Dr. Oliver Smithies, who developed homologous recombination technology in mouse ES cells. As a result of these technical advances, functional analysis of genes has progressed considerably using mice with gene knock-outs or other genetic modifications.

It is well known that mouse cells can be immortalized simply by continuous *in vitro* culture, for example, using the so-called "3T3 protocol". One widely exploited example of an immortalized cell line is NIH3T3, which continues to be used in a wide range of experiments. In contrast, it is not possible to immortalize human somatic cells in a similar manner and this difficulty gave rise to the widespread assumption that it would not be possible to establish human ES cell lines. However, in 1998, 17 years after the first establishment of mouse ES cell lines, it was reported that human ES cell lines could also be produced by continuous *in vitro* culture[4].

Therapeutic cloning

The ability to reprogram mammalian somatic cells by nuclear transfer and to establish human ES cell lines stimulated medical scientists to investigate the creation of ES cell lines using nuclear transfer as a potential means of achieving "therapeutic cloning". If this technology could be established as a viable therapy, then patients who would benefit from somatic cell transplantation could be treated with nuclear-transferred ES cells produced using their own somatic cells, which would avoid the possibility of transplant

rejection as the cells possess the same major histocompatibility (MHC) antigens as host tissue.

Although an earlier report of successful therapeutic cloning by a group in Korea proved false, it was recently reported that primate ES cell lines have been established by nuclear transfer technology[5]. Since unfertilized primate eggs are much more fragile than those of rodents, it may still take some time to establish the technology for use in human therapeutic cloning. However, the prospect of using such therapy no longer seems to be so distant.

A search for alternative technologies to therapeutic cloning

An important limitation to the use of therapeutic cloning is that it requires unfertilized eggs. Human eggs are very difficult to obtain and, moreover, their use for this purpose also raises serious ethical issues. For these reasons, a search has been initiated for alternative methodologies that avoid nuclear transfer. One approach has been to search for factors in unfertilized eggs that may be required for the reprogramming of transferred somatic nuclei. Another avenue of research has been to elucidate which genes specifically function in ES cells, since these genes may maintain the undifferentiated state of ES cells and thus might be able to induce reprogramming of nuclei in terminally differentiated somatic cells.

The research group led by Dr. Shinya Yamanaka reported the first success in the latter approach. They were able to induce differentiated mouse somatic cells to become pluripotent stem cells by application of four defined factors6. The enforced expression of the transcription factors Oct3/4, Sox2, Klf4 and c-Myc in terminally differentiated somatic cells induced cellular reprogramming and changed the cells into ES-like pluripotent stem cells. These reprogrammed cells were named “induced pluripotent stem (iPS) cells”. Subsequently, in the year after establishment of human iPS cell lines was first reported, several other groups also succeeded with this methodology[7-10]. Dr. Shinya Yamanaka, who developed the method, was given the Albert Lasker Basic Medical Research Award in 2009 together with Dr. John Gurdon.

The mechanisms underlying the reprogramming of terminally differentiated somatic cells following the enforced expression of the four factors remain to be elucidated. It is now known that expression of these factors after exogenous introduction is completely suppressed in established iPS cells. Thus, the factors seem to be required only for the reprogramming process but not for maintenance of pluripotency. Regardless of the mechanisms involved, this discovery clearly indicated that terminally differentiated somatic cells could be reprogrammed without nuclear transfer into unfertilized eggs and opened a new dawn for therapeutic cloning[11-13].

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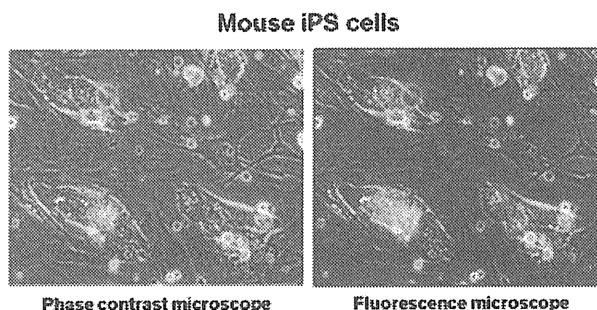
Figure 1



A mouse iPS cell line, iPS-MEF-Ng-20D-17, established by Dr. Yamanaka's group, that can differentiate into germ line cells. In this cell line, GFP has been knocked-in under control of the Nanog promoter and thus the undifferentiated cells express GFP.

Progress in methods to establish iPS cells

Although the first mouse iPS cell lines could not differentiate into germ line cells, the next generation of iPS cell lines was able to undergo this differentiation process (Figure 1)[14]. It has also been reported that c-Myc is not necessary for establishing iPS cells, although the efficiency of reprogramming was reduced in its absence15. Use of c-Myc will probably not be necessary in future as methodological improvements have

continuously increased the efficiency of cellular reprogramming[16-24].

The first method for establishing iPS cells utilized a retroviral vector to introduce the four factors. Since retroviral vectors integrate into the genome, it is possible that they might affect the function of endogenous genes and thus it might be inappropriate to make use of such cells in the clinic. The group of Dr. Yamanaka developed an alternative approach that avoided the use of retroviral vectors by employing ordinary plasmid vectors[25]. Yu *et al.* also reported a method for establishing iPS cell lines that obviated the need for vector and transgene sequences[26].

Other approaches for producing iPS cell lines have recently been described[16-24]. One of the most promising is that utilizing the Sendai virus vector. As the Sendai virus does not integrate into the host genome, it is possible to create iPS cell lines that do not carry the exogenous reprogramming factors[27]. Of course, since the mechanisms involved in reprogramming somatic cells remain uncertain, we cannot be certain that iPS cells produced with the Sendai virus vector possess identical characteristics to ES cells. However, it is highly likely that they will be more suitable for clinical applications than cells with integration of the exogenous genes.

Standardization of iPS cells

The characteristics of ES cells vary among different cell lines. Indeed, even those of mouse ES cell lines derived from an inbred mouse strain show significant differences; for example, some cell lines can differentiate into germ line cells whereas others cannot. It is therefore unsurprising that the characteristics of human ES cell lines also differ among cell lines. Additionally, the genomic backgrounds of human ES cell lines differ among cell lines. As mentioned above, the mechanisms of reprogramming are uncertain and, therefore, standardization of human iPS cell lines is currently a very difficult process. It is clear that conventional analyses, such as detection of molecular markers of the undifferentiated state or observation of teratoma formation in immunodeficient mice, are insufficient to characterize iPS cells and that more objective and quantitative analyses are required. Gene expression profiling analysis and epigenetic modification analysis are candidate methods for characterizing iPS cell lines. Given the diversity in human genetic backgrounds, characterization of human iPS cell lines will require objective and quantitative analyses of at least a few hundred cell lines.

Needless to say, fundamental quality controls of the cell lines are also essential. First, the cell lines should be free of microbial contamination. Bacterial or fungal infections are less important as these are readily detected. However, mycoplasmal infections are less easy to identify without examination of the cultures since the majority of cell lines will survive the presence of mycoplasma. Second, misidentification of cell lines should be eliminated. Profiling of short tandem repeat (STR) polymorphisms is a very useful means of ensuring the correct identification of cell lines and is now routinely performed in major cell banks around the world[28, 29].

Clinical grade stem cells

Cells derived from ES or iPS cell cultures have applications in many fields of medical science. For example, liver or heart cells derived from stem cells can be used for pharmaceutical drug screening. Needless to say, however, the most attractive application is for clinical therapies. Such applications require so-called clinical grade stem cells. What are the characteristics of clinical grade stem cells?

There are many risks involved in using long-term cultured cell lines in the clinic[30]. The most critical of these risks is tumorigenicity. Since both ES and iPS cells are immortalized, in one sense they are very close in nature to tumor cells. Indeed, they can give rise to teratomas when transplanted into immunodeficient mice. Although these teratomas are not malignant teratocarcinomas, they are nevertheless tumors.

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One way to make use of stem cell-derived cells is to induce post-mitotic cells that are unable to proliferate. Terminally differentiated neural and heart cells are possible candidates for post-mitotic cells. Enucleated cells (cells that do not possess nuclei), such as red blood cells and platelets, are absolutely free of risk of tumorigenicity and may therefore be close to use for clinical applications, although systems for mass production of such cells need to be established prior to their application.

Disease-specific iPS cells

The ability to produce iPS cell lines will be benefit both regenerative medicine and also research into a variety of diseases[31-39]. For example, it is currently not possible to obtain neural cells from patients suffering from neural diseases. However, iPS cell lines could be established from their somatic cells, such as skin fibroblasts, and neural cells could then be derived in culture. Such iPS-derived neural cells could be subjected to a range of research analyses, such as investigation of the underlying mechanisms of disease or for drug discovery for disease therapy.

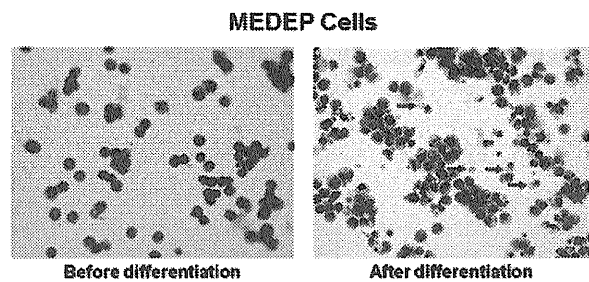
There are still many diseases for which the causes are unknown and therapies have not been developed. Thus, the value of disease-specific iPS cells should increase tremendously in the near future. Human cancer cells are exceptional in that they can be immortalized simply by continuous *in vitro* culture, and many cancer cell lines are currently used for experimental analyses. Of note, however, low malignancy and benign tumor cells cannot be immortalized in a similar fashion to normal human somatic cells. Therefore, iPS cells derived from low malignancy or benign tumor cells may also be useful for the study of such diseases.

Establishment of progenitor cell lines from iPS cells

Immortalized cell lines that possess the ability to differentiate are very valuable not only for analyzing the mechanisms of differentiation but also for the provision of abundant differentiated and mature cells. For example, immediate progenitor cell lines of red blood cells have been established from mouse ES cells[40], and these cell lines can produce mature enucleated red blood cells *in vitro* (Figure 2). If similar progenitor cell lines can be established from human ES or iPS cells, then they may be of use for the *in vitro* production of transfusable red blood cells.

Figure 2

MEDEP Cells



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Mouse ES cell-derived erythroid progenitor (MEDEP) cells before (left) and after (right) in vitro differentiation. Arrowheads in the right photograph indicate enucleated red blood cells.

With regard to mature cells such as liver or heart cells, immediate progenitor cell lines for these cells will be invaluable. Such progenitor cell lines could be established using human ES or iPS cells in a similar manner as for the establishment of red blood cell progenitor cell lines from mouse ES cells.

New insights into cell plasticity

It is possible that the reprogramming of somatic cells is incomplete, that is, that the cells are not fully reprogrammed. Such partially reprogrammed cells might be termed "pseudo iPS cells". What characteristics might distinguish such cells? As they are highly likely to be progenitor cells for specific somatic cells, we might be able to obtain progenitor cell lines using these "pseudo iPS cells" without recourse to genuine iPS cells that have been fully reprogrammed. In this context, a highly instructive paper was published recently. Vierbuchen *et al.* reported that they had succeeded in directly converting fibroblasts into functional neurons by induced

expression of three transcriptional factors[41]. By utilizing cell plasticity, it may be feasible to obtain various types of somatic cells without making genuine iPS cells.

Epilogue

We now have an excellent technology for the relatively simple production of ES-like pluripotent stem (iPS) cells from somatic cells. A recent report indicates that iPS cell lines can be established even from peripheral blood cells. Since obtaining a blood sample is a commonplace clinical activity, development of iPS cell lines will undoubtedly spread rapidly through use of peripheral blood cells, particularly for preparation of disease-specific iPS cell lines.

In future, it is possible that every person will have their own iPS cell lines, prepared when they were still healthy, for future applications in clinical examination and/or therapy. Is this just science fiction? I believe not.

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UICC
global cancer control

Database of misidentified cell lines

R. Ian Freshney

Center for Oncology and Applied Pharmacology, Division of Cancer Sciences and Molecular Pathology, University of Glasgow, Glasgow, Scotland, United Kingdom

Dear Sir,

Much of current cancer and cell biology research depends on the use of cell lines cultured from normal and malignant tissue. However, ever since the time when continuous cell lines were first established, there has been a problem of the more vigorous lines contaminating and overgrowing more slowly growing cultures. This has been compounded by confusion of one cell line with another by mislabeling in routine culture or during and after cryopreservation. The result is that some 15–20% of cell lines in current use may not be what they are claimed to be. This has prompted a number of recent reports in the literature[1–7] and discussions at scientific meetings. One of the main conclusions is that there needs to be a way to alert scientists using established and frequently propagated cell lines that there is a significant risk that they may be using cell lines which are not what they need them to be. This issue of International Journal of Cancer will address this problem and wants to increase the awareness of authors submitting their work for publication and of reviewers considering the merit of the work. Restrictions and conditions will be imposed regarding proof of authentication of cell lines used and advice given on how to authenticate cell lines (see editorial and letter by W. Dirks). My purpose in this letter is to notify the scientific community of the existence and free availability of a list of cell lines which are known or suspected to be falsely identified or cross contaminated. This will allow scientists embarking on a project or reviewers considering the work for publication, to have access to a data source which will advise them on the respective cell line's authenticity. This list is available for download from: http://www.hpacultures.org.uk/services/celllineidentityverification/misidentifiedcelllines.jsp by following the link after my and Amanda Capes-Davis's names. It has been compiled from quality assurance carried out by a number of cell banks (ATCC, CellBank Australia, sDSMZ, ECACC, JCRB, and RIKEN) and published on their websites, from an entry in Wikipedia, and from reports in the scientific literature. It must be emphasized that while many of the cell lines listed are clearly and incontrovertibly not what they are supposed to be, original and authentic stocks of other lines may yet exist. Where this is believed to be the case the line is included in the second table. This list will be published (Capes-Davis *et al.*, ms in preparation).

I would request that anyone who uses this list and finds that some misidentified cell lines have been omitted or that some cell lines reported as misidentified do have authentic stocks available should contact me (i.freshney@ntlworld.com), and I will arrange to have the database updated.

The recommended procedure for anyone contemplating the use of cell lines is as follows:

- Check that the cell line that you intend to use is not listed in the above database.
- Ensure that the cell line is obtained from a properly authenticated source (and that may not be the originator), preferably from one of the recognized cell banks.
- Authenticate cell lines received from a nonauthenticated source on receipt (see letter of W. Dirks, this issue, and instruction for authors of IJC).
- Repeat authentication at intervals of 3–6 months for cell lines used for an extended study, before cryopreservation, and after thawing for further use.

It may not be possible to eliminate misidentification entirely, as new examples will continue to appear, but following these precautions should reduce the frequency and minimize the spread of the problem.

Yours sincerely,
R. Ian Freshney

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Letter to the Editor

Cell line cross-contamination initiative: an interactive reference database of STR profiles covering common cancer cell lines

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[3] JCRB—Japanese Collection of Research Bioresources, Osaka, Japan
[4] ATCC—American Type Culture Collections, Manassas, VA

Dear Sir,

Recent reports[1–4] demonstrate the growing perception in the scientific community that cross contamination (CC) of mammalian cell lines represents a major risk for generating false scientific data. The level to which research has been compromised by the use of contaminated or misidentified cell lines has become a major concern for scientists, granting agencies, and, increasingly, scientific journals. In 2007, a group of cell biologists led by Roland M. Nardone petitioned the United States Secretary of Health and Human Services to develop an active program for cell line authentication.[5] They stressed that research and teaching tools in diverse fields of science and industry would be unimaginable without cell cultures. Despite the key importance of cell cultures, only little consensus exists regarding the technical means by which cell line identity can be controlled and how to follow through the results of any such testing.

The key problems of CC are known and chronic in nature: neglecting guidelines for quality control and disregarding adequate cell culture techniques are the main reasons why cell lines have been misidentified or cross contaminated. The incidence of CC in directly and indirectly provenanced cell lines alike[1,3] implies that the majority of false cell lines are perpetrated in originators' own laboratories, presumably by failures during the establishment of new cell lines. A plethora of reports unmasking bogus cancer cell lines, including members of the NCI-60 panel used to generate reference baseline transcriptional drug responses has triggered calls for remedial action.[5,6] Nevertheless, standard authentication procedures for testing cell line identity have yet to be defined.

Short tandem repeat (STR) microsatellite sequences are highly polymorphic in human populations, and their stability throughout the lifespan of individuals renders STR profiling (typing) ideal for forensic use. STR typing has served as a reference technique for identity control of human cell lines at Biological Resource Centers (BRCs) since the turn of the millennium.[7] Ideally, authentication involves coincident STR typing of paired donor and derived cell line samples. However, this ideal is met by a few recently established cell lines only. Most widely used cell lines are decades old and their identification is largely retrospective and multidisciplinary, combining diverse criteria such as uniqueness and the congruence of STR profiles across independent samples with the consistency of observed karyotypes with those reported by the originators.

The DSMZ as well as the ATCC, JCRB, and RIKEN repositories have generated large databases of STR cell line profiles. By using the same microsatellite loci at these BRCs, individual databases could be merged, thereby facilitating interactive searches. This work was piloted at the DSMZ to generate an international reference STR profile database for human cell lines. To render it user friendly, a simple search engine for interrogating STR cell line profiles has now been made available on the homepages of JCRB and DSMZ (http://cellbank.nibio.go.jp/cellbank_e.html, http://www.dsmz.de/STRanalysis). Registered users simply login at the search-site on the DSMZ homepage and will be guided. Aided by simple prompts, users can input their own cell line STR data to retrieve best matches with authenticated cell lines listed on the database.

Once the problem of false negatives due to discrepant representation of single STR alleles, *e.g.*, by losses of heterozygosity and bottlenecking selection—has been tackled and unambiguous search results are produced, human cell lines will need to be consistent with consensus STR reference data sets. STR profiles of all human cell lines distributed by DSMZ, JCRB, and RIKEN and one-third of the cell lines distributed by ATCC are now publicly accessible on interactive databases where match criteria have been arbitrarily set to 95%. Inevitably, reference profiles remain subject to revision until all commonly held cell lines have been STR typed across participating repositories. At present, about 2,342 such cell lines have been STR typed and are represented as reference sets on the database.

The authors of this article are currently participating in an international workgroup organized by the ATCC Standards Development Organization, (ATCC SDO) to develop a standardized methodology (protocols and procedures for STR analysis) for authenticating human cell lines. An additional

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goal of the workgroup is to establish a global database for STR profiles of human cell lines. The development of the consensus standard offers a new tool to the cell biology community that will foster reproducibility and comparability of cell lines used in different laboratories. Armed with these tools, online verification of cell line identity should prove a vital weapon to combat the havoc of cell line cross contamination which has dogged cancer research since inception.

Yours sincerely,
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NBRP databases: databases of biological resources in Japan

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ABSTRACT

The National BioResource Project (NBRP) is a Japanese project that aims to establish a system for collecting, preserving and providing bioresources for use as experimental materials for life science research. It is promoted by 27 core resource facilities, each concerned with a particular group of organisms, and by one information center. The NBRP database is a product of this project. Thirty databases and an integrated database-retrieval system (BioResource World: BRW) have been created and made available through the NBRP home page (http://www.nbrp.jp). The 30 independent databases have individual features which directly reflect the data maintained by each resource facility. The BRW is designed for users who need to search across several resources without moving from one database to another. BRW provides access to a collection of 4.5-million records on bioresources including wild species, inbred lines, mutants, genetically engineered lines, DNA clones and so on. BRW supports summary browsing, keyword searching, and searching by DNA sequences or gene ontology. The results of searches provide links to online requests for distribution of research materials. A circulation system allows users to submit details of papers published on research conducted using NBRP resources.

INTRODUCTION

Japanese bioresources for life science research have an 80 year history. Although some unique and precious collections of resources have been accumulated during this time, some of these have recently became dispersed and lost as a result of the increasing age of their providers or through shortages of funds, so that systems for providing useful collections of resources became inadequate. To improve this situation, it was necessary to establish a sustainable environment in which researchers could readily obtain bioresources, so the National BioResource Project (NBRP) began in 2002. The species included in the project were selected on the basis that they were indigenous to Japan or that they were model organisms currently studied or expected to be studied in the future by large numbers of researchers.

A major feature of this project is that it promotes the centralization of resources and information to ensure continuity. Resources are organized by species or groups of organisms, and a system of interaction between the resource and the information center [which belongs to the National Institute of Genetics (NIG)] was created to centralize the information. All the information in the databases is publicly available and several enhancements

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