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—Review—
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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.

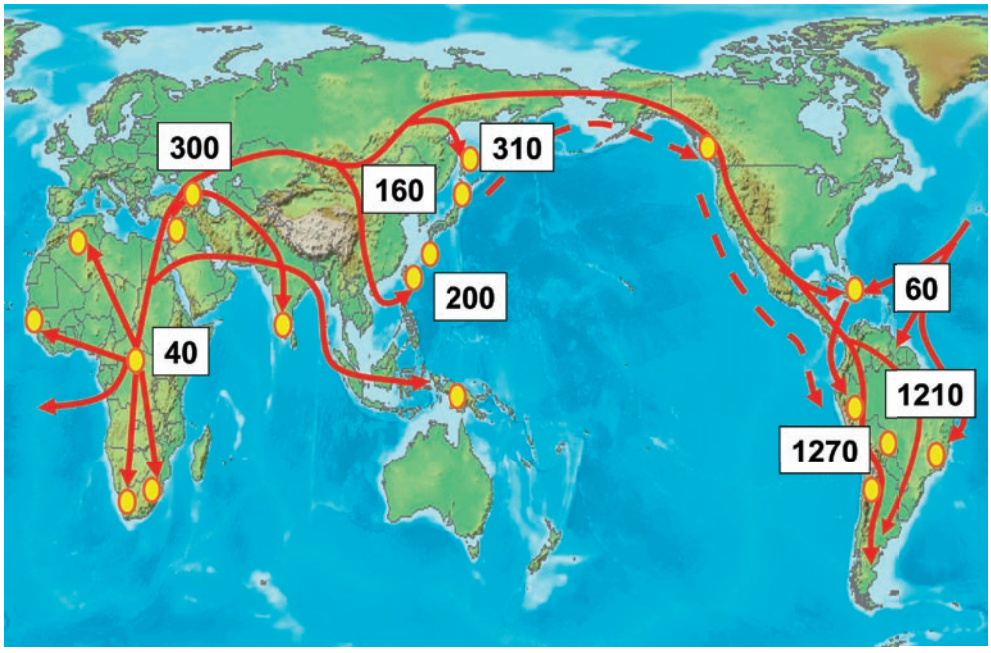

Fig. 1. Sonoda-Tajima collection. This collection contains a great number of cells derived from many people from various areas around the world. The numbers in the figure indicate the sample numbers collected in each area.

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.

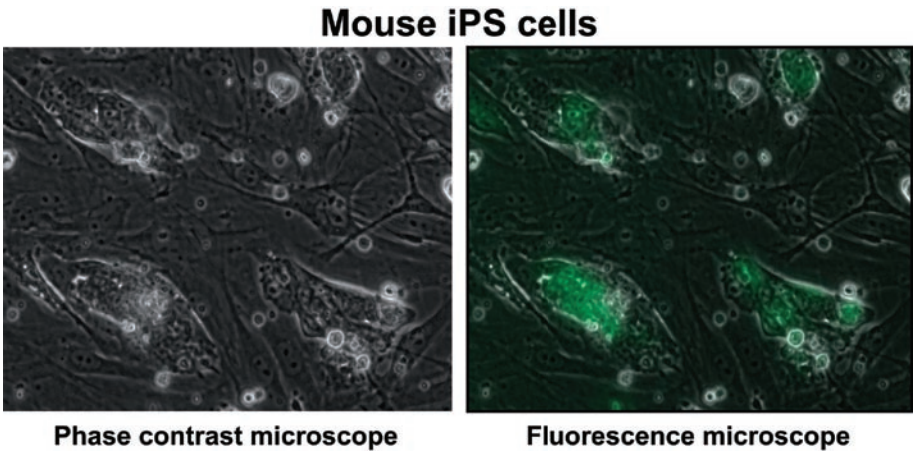

Fig. 2. Mouse iPS cells deposited by Dr. Yamanaka. GFP gene is knocked-in under Nanog promoter, and thus, the expression of GFP can be detected in undifferentiated cells.

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.

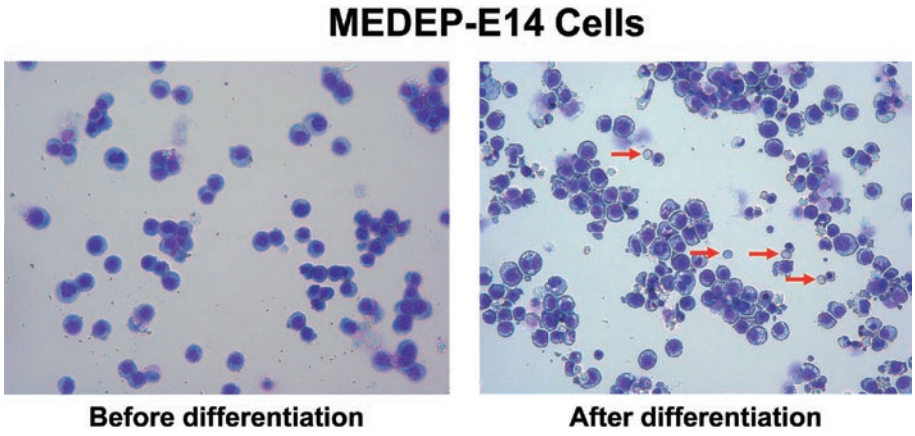

Fig. 4. An erythroid cell line established from mouse ES cells (E14), MEDEP-E14. After induction of differentiation, enucleated red blood cells can be detected. Red arrows indicate enucleated red blood cells.

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.

cellqa@brc.riken.jp

E-mail address for questions regarding deposit and donation of cell lines is as follows.

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