Cell Engineering Division

Head

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Goal

The Cell Engineering Division of RIKEN BioResource Center is a nonprofit public "cell bank" that accepts donations and deposits of human and animal cell materials developed by the life science research community. It examines, standardizes, amplifies, preserves, and provides cell materials to interested scientists around the world.

The major cell materials used around the world have been cultured cell lines, i.e., immortalized clone 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.

Stem cell lines such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are of great value in current biology and medical sciences. Thus, we are extensively collecting such stem cell lines, aiming to contribute to such fields as developmental biology, transplantation medicine, and regenerative medicine.

On the other hand, the demand for non-immortalized cells has recently increased. To meet this demand, we have recently started the banking of primary human cells including somatic stem cells, such as human umbilical cord blood cells and primary cultured human mesenchymal cells.

The staffs 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 cell lines and the development of new technology to utilize cell materials.

History

RIKEN Cell Bank was established in 1987 when a committee of scientists recognized the need for a central collection of animal cell materials that would serve scientists. In 2001, RIKEN BioResource Center (RIKEN BRC) was established and then Cell Bank was reorganized into the Cell Engineering Division. In 2002, the division 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.

Activities Relating to Cell Banking

1. Cell Banking: Human and animal cell lines
2. Quality control
3. Ethical matter relating to human cell materials
4. Human embryonic stem (ES) cells
5. Induced pluripotent stem (iPS) cells
6. Human somatic stem cells: Umbilical cord blood cells and mesenchymal stem cells
7. Human B cells derived from healthy volunteer and people suffering from some diseases
8. Sonoda-Tajima collection: B cells derived from various human species and tribes
9. Goto collection: Fibroblasts and B cells derived from patients suffering from Werner syndrome

Activities Relating to Development of New Cell Resources

1. Technological development for the culture, quality control, preservation and differentiation of embryonic stem (ES) cells
2. Technological development for the establishment, culture, quality control, preservation and differentiation of induced pluripotent stem (iPS) cells
3. Technological development for the establishment of differentiated cell lines from ES cells and iPS cells
4. Technological development for the improvement of cell culture

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Activities Relating to Cell Banking

1. Cell Banking: Human and animal cell lines (Fig. 1)

Cell lines are very useful materials in all fields of biology and they have been utilized in many kinds of experiment. We have approximately two thousand cell lines, of which approximately 1,300 lines are immediately available for distribution. Approximately half of the cell lines were derived from human cancer cells. These cancer tissue-derived cell lines are very valuable for oncology and tumor immunology. Another half of the cell lines were derived from animals. In addition to animal cell lines derived from somatic cells, many kinds of mouse embryonic stem (ES) cell line including C57BL/6-derived ES cell lines are also available. In recent years, around four thousand ampoules have been distributed annually, mostly to nonprofit organizations (80%) and approximately 10% overseas. We will continue to accept deposits and donations of cultured animal cell lines and expand the collection, since the significance of the mentioned cell lines in the field of biology will never cease.

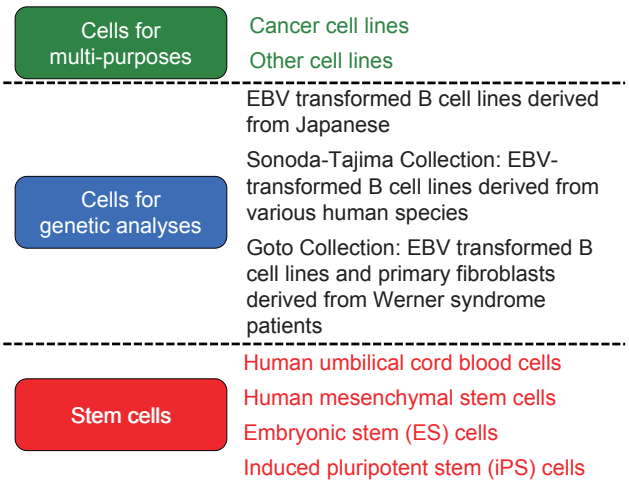

Figure 1. The cell materials that RIKEN BRC is providing with.

2. Quality control (Fig. 2)

Cross-contamination between cultured cell lines can result in the generation of erroneous scientific data. Hence, it is very important to eliminate cell lines whose origin differs from that claimed. Interspecies contamination can be detected by various established methods, such as karyotype and isozyme analyses. However, it is impossible to detect intraspecies cross-contamination unless a technology for detecting differences between cell lines at the molecular level is developed. Recently, the profiling of short tandem repeat (STR) polymorphisms (STR profiling) has been established as a method of analyzing gene polymorphism. STR profiling is a simple and reliable method of identifying individual cell lines. All human cell lines that we are currently distributing were analyzed by STR profiling to authenticate its identity. We found through such analysis that nearly 10% of the deposited cell lines were misidentified. Thus, we conclude that 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.

In relation to the cell lines derived from mice, we are performing simple sequence length polymorphism (SSLP) analysis to confirm the mouse strain origin. Similarly to the human cell lines, several mouse cell lines were found to be misidentified.

We have established a quality management system (QMS) and all works in our laboratory are performed according to this QMS. Our QMS has been accredited by ISO 9001:2000, July 2007 (Fig. 3).

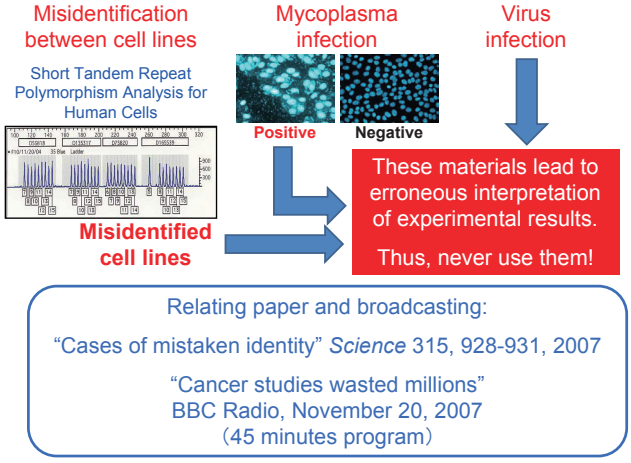

Figure 2. Quality control of cell materials.
Approximately 10% of the cells deposited in RIKEN BRC were misidentified. In addition, approximately 30% of the cells deposited in RIKEN BRC were infected by mycoplasma.

Quality management system (QMS) according to the required matters of ISO 9001:2000.

Major cell banks around the world such as DSMZ (Germany) and ATCC (U.S.A.) have also been accredited by ISO 9001:2000 for their QMS.

Figure 3. The cell engineering division of RIKEN BRC has been accredited by ISO 9001:2000 for the quality management system (QMS) performed in the division.

3. Ethical matter relating to human cell materials

The cell banking of human cells requires strict regulation about ethical matters. We only accept human cell donations that are approved by the Institutional Review Board (IRB) at RIKEN Tsukuba Institute. Furthermore, RIKEN BRC contracts the Material Transfer Agreement (MTA) with the organization that deposits or donates human cells to RIKEN BRC. In the MTA, RIKEN BRC confirms that the human cell resource was 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, it also contracts the MTA with the user. As for certain human cells, such as umbilical cord blood, an approval by the IRB of the user organization is also required.

4. Human embryonic stem (ES) cells

In April 2008, the Ministry of Education, Culture, Sports, Science and Technology of Japan approved that our center collects and distributes human ES cell lines. 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 already accepted the deposit of three human ES cell lines established at Kyoto University (khES-1, khES-2, khES-3). We are currently preparing for their distribution.

5. Induced pluripotent stem (iPS) cells (Fig. 4)

Dr. Shinya Yamanaka of Kyoto University (Japan) has developed a breakthrough technique in the field of biology. He enabled the induction of pluripotent stem cells from somatic cells using some defined factors (Oct3/4, Sox2, Klf4, c-myc). We have accepted the deposit of a mouse iPS cell line from Dr. Yamanaka and have already distributed it to more than 100 institutions thus far. In addition, we have also accepted the deposit of human iPS cell lines from Dr. Yamanaka. Thus, the number of iPS cell lines derived from normal cells as well as from cells with genetic mutations will increase rapidly. Of course, we will also extensively collect iPS cell lines established around the world.

6. Human somatic stem cells: Umbilical cord blood cells and mesenchymal stem cells

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 usually discarded if they are not used in transplantation. Provided that the mother of a newborn baby agrees to allow the use of her umbilical cord blood cells in research, the material can provide a valuable resource without the complicating factor of ethical concerns. By collaborating with the "Japanese Cord Blood Bank Network", we are supplying human umbilical cord blood to domestic researchers in order to contribute to the fields of transplantation and regenerative medicine.

Compared with primary cells derived from experimental animals, human primary cells are very difficult to obtain. Current research in life science, however, requires human primary cells, such as stem cells, particularly in the fields of transplantation and regenerative medicine. We succeeded in establishing a system for providing such human primary cells efficiently. By collaborating with researchers who developed technologies for expanding human mesenchymal stem cells *in vitro* very efficiently, we are supplying human mesenchymal stem cells to researchers. Mesenchymal stem cells can differentiate to bones and cartilage and reportedly also to neurons and cardiomyocytes.

7. Human B cells derived from healthy volunteers and people suffering from some diseases

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 are collecting human B cell lines immortalized by Epstein-Barr virus transformation. The donors of B cells are not only healthy volunteers but also volunteers with certain diseases.

8. Sonoda-Tajima collection: B cells derived from various human species and tribes

This is a collection of B cell lines derived from many human species and tribes around the world, mainly old-mongoloid individuals living in South America. The B cell lines have been transformed by Epstein-Barr virus transformation. The collection is very useful for the research of human genetics.

9. Goto collection: Fibroblasts and B cells derived from patients with Werner syndrome

This is a collection of cells derived from patients with Werner syndrome. Werner syndrome is characterized by the premature appearance of features associated with normal aging and cancer predisposition. Most patients with Werner syndrome in the world are Japanese. Thus, many scientists around the world are focusing on this collection.

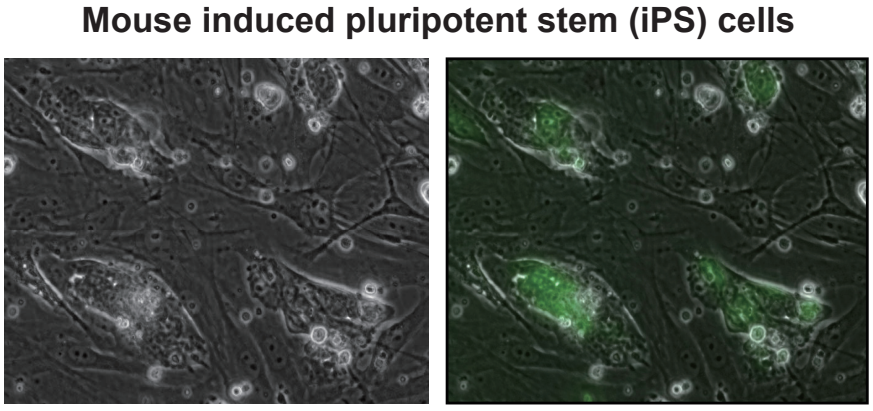

Figure 4. Mouse induced pluripotent stem (iPS) cells.
Green fluorescent protein (GFP) is expressed driven by the Nanog promoter activity (right photo).

Activities Relating to Development of New Cell Resources

1. Technological development for culture, quality control, preservation and differentiation of embryonic stem (ES) cells

Embryonic stem cells have a high potential for use in the fields of basic biology and applied science. We are developing technologies for the following: (1) an efficient culture method on a large scale, (2) an efficient quality control method, (3) an efficient preservation method, and (4) an efficient differentiation method.

2. Development of technology for establishment, culture, quality control, preservation and differentiation of induced pluripotent stem (iPS) cells

We are currently trying to establish human iPS cell lines from fibroblast-like cells. After establishing human iPS cell lines, we will distribute those cell lines to scientists around the world. In addition, we are developing technologies for the same themes described in the ES cell section.

3. Technological development for establishing differentiated cell lines from ES cells and iPS cells

Human and animal cell lines with multipotency or tissue-specific feature 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 stem cells may lead to the establishment of such cell lines by immortalizing 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. 5). Third, the reprogramming of somatic cells may lead to the establishment of such cell lines except iPS cells. In fact, in establishing the iPS cell line, various cells that are not transformed into so-called iPS cells emerge. We are investigating the possibilities of these phenomena.

4. Technological development for improving cell culture

All kinds of cell 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 in this field.

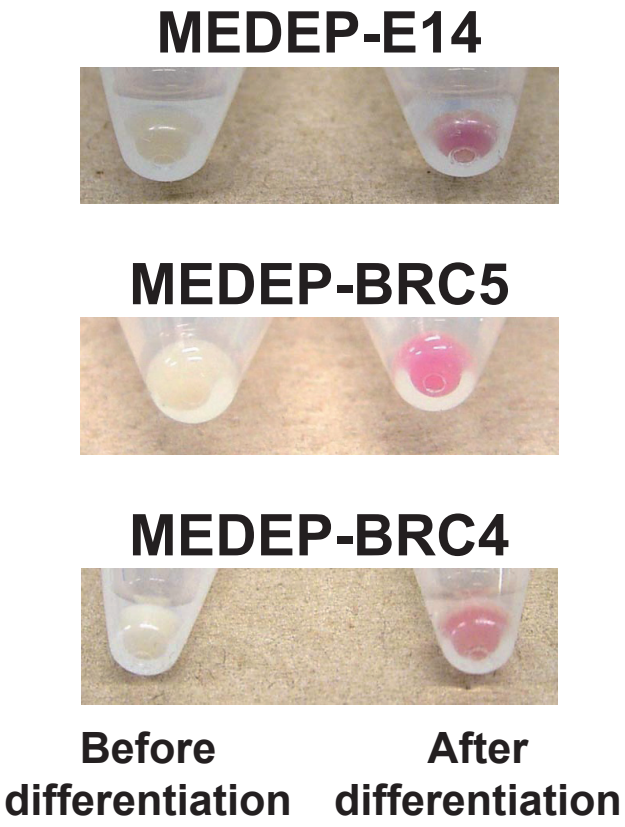

Figure 5. Mouse erythroid cell lines derived from mouse ES cells (MEDEP-E14, MEDEP-BRC5, and MEDEP-BRC4).
The three cell lines can differentiate into mature erythroid cells and produce hemoglobin. Thus, the cell pellet of each cell line changes to red after in vitro differentiation.

Publications

【Original Papers】(*Peer reviewed journals)

1. Tamagawa T., Ishiwata I., Nakamura Y.: "Differentiation of human amniotic membrane cells into osteoblasts in vitro." Hum. Cell 18: 191-195 (2005).*

2. Nagano Y., Matsui H., Muramatsu M., Shimokawa O., Shibahara T., Yanaka A., Nakahara A., Matsuzaki Y., Tanaka N., Nakamura Y.: "Rebamipide significantly inhibits indomethacin-induced mitochondrial damage, lipid peroxidation, and apoptosis in gastric epithelial RGM-1 cells." Dig. Dis. Sci. 50: 76-83 (2005).*

3. Miharada K., Hiroyama T., Sudo K., Nagasawa T., Nakamura Y.: "Lipocalin 2 functions as a negative regulator of red blood cell production in an autocrine fashion." FASEB J. 19: 1881-1883 (2005).*

4. Miharada K., Hiroyama T., Sudo K., Nagasawa T., Nakamura Y.: "Refinement of cytokine use in the in vitro expansion of erythroid cells." Hum. Cell 19: 30-37 (2006).*

5. Yoshino K., Iimura E., Saijo K., Iwase S., Fukami K., Ohno T., Obata Y., Nakamura Y.: "Essential role for gene profiling analysis in the authentication of human cell lines." Hum. Cell 19: 43-48 (2006).*

6. Hiroyama T., Miharada K., Aoki N., Fujioka T., Sudo K., Danjoh I., Nagasawa T., Nakamura Y.: "Long lasting in vitro hematopoiesis derived from primate embryonic stem cells." Exp. Hematol. 34: 760-769 (2006).*

7. Miharada K., Hiroyama T., Sudo K., Nagasawa T., Nakamura Y.: "Efficient enucleation of erythroblasts differentiated *in vitro* from hematopoietic stem and progenitor cells." Nat. Biotechnol. 24: 1255-1256 (2006).*

8. Hasegawa K., Fujioka T., Nakamura Y., Nakatsuji N., Suemori H.: "A method for the selection of human embryonic stem cell sub-lines with high replating efficiency after single cell dissociation." Stem Cells 24: 2649-2660 (2006).*

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10. Sudo K., Kanno M., Miharada K., Ogawa S., Hiroyama T., Saijo K., Nakamura Y.: "Mesenchymal progenitors able to differentiate into osteogenic, chondrogenic, and/or adipogenic cells in vitro are present in most primary fibroblast-like cell populations." Stem Cells 25: 1610-1617 (2007).*

11. Tamagawa T., Oi S., Ishiwata I., Ishikawa H., Nakamura Y.: "Differentiation of mesenchymal cells derived from human amniotic membranes into hepatocyte-like cells *in vitro*." Hum. Cell 20: 77-84 (2007).*

12. Hiroyama T., Sudo K., Aoki N., Miharada K., Danjoh I., Fujioka T., Nagasawa T., Nakamura Y.: "Human umbilical cord-derived cells can often serve as feeder cells to maintain primate embryonic stem cells in a state capable of producing hematopoietic cells." Cell Biol. Int. 32: 1-7 (2008).*

13. Miharada K., Hiroyama T., Sudo K., Danjoh I., Nagasawa T., Nakamura Y.: "Lipocalin 2-mediated growth suppression is evident in human erythroid and monocyte/macrophage lineage cells." J. Cell. Physiol. 215: 526-537 (2008).*

14. Hiroyama T., Miharada K., Sudo K., Danjoh I., Aoki N., Nakamura Y.: "Establishment of mouse embryonic stem cell-derived erythroid progenitor cell lines able to produce functional red blood cells." PLoS ONE 3: e1544 (2008).*

15. Katayama H., Yamane Y., Furukawa Y., Kitagawa S., Nakamura Y., Yoshino K.: "Activation of focal adhesion

kinase in detached human epidermal cancer cells and their long-term survival might be associated with cell surface expression of laminin-5." Acta Derm. Venereol. 88: 100-107 (2008).*

16. Mitsuoka K., Miyoshi S., Kato Y., Murakami Y., Utsumi R., Kubo Y., Noda A., Nakamura Y., Nishimura S., Tsuji A.: "Cancer detection using a PET tarcer, 11C-Glycylsarcosine, targeted to H^+/peptide transporter." J. Nucl. Med. 49: 615-622 (2008).*

17. Tamagawa T., Ishiwata I., Ishikawa H., Nakamura Y.: "Induced in vitro differentiation of neural-like cells from human amnion-derived fibroblast-like cells." Hum. Cell 21: 38-45 (2008).*

18. Yamanaka Y., Heike T., Kumada T., Shibata M., Takaoka Y., Kitano A., Shiraishi K., Kato T., Nagato M., Okawa K., Furushima K., Nakao K., Nakamura Y., Taketo M.M., Aizawa S., Nakahata T.: "Loss of Borealin/DasraB leads to defective cell proliferation, p53 accumulation and early embryonic lethality." Mech. Dev. 125: 441-450 (2008).*

19. Takayama N., Nishikii H., Usui J., Tsukui H., Sawaguchi A., Hiroyama T., Eto K., Nakauchi H.: "Generation of functional platelets from human embryonic cells in vitro via ES-sacs, VEGF-promoted structures that concentrate hematopoetic progenitors." Blood 11: 5298-5306 (2008).*

Oral Presentations

【International Conferences】

1. Hasegawa K., Fujioka T., Nakamura Y., Nakatsuji N., Suemori H.: "Isolation of human embryonic cell lines showing high cloning efficiency." International Symposium on Germ Cells, Epigenetics, Reprogramming and Embryonic Stem Cells, Kyoto, Japan, Nov. (2005).

2. Nakamura Y.: "Role of lipocalin 2 in erythropoiesis." 15th Annual Growth Factor and Signal Transduction (GFST) symposium, Iowa, USA, Jul. (2006).

3. Takayama N., Nishikii H., Hiroyama T., Nakamura Y., Tsukui H., Eto K., Nakauchi H.: "Human embryonic stem cell-derived "NET-Link" structure serves as a hematopoietic progenitor niche and favors generation of mature megakaryocytes and functional platelets." The American Society of Hematology, 48th Annual Meeting and Exposition, Orlando, USA, Dec. (2006).

4. Miharada K., Hiroyama T., Sudo K., Nakamura Y.: "Regulation of red blood cell number by Lipocalin 2, that inhibits erythroblast differentiation and proliferation." ISEH 36th Annual Scientific Meeting, Hamburg, Germany, Sep. (2007)

5. Sudo K.: "Mesenchymal progenitors able to differentiate into osteogenic, chondrogenic, and/or adipogenic cells in vitro are present in most primary fibroblast-like cell populations"World Congress on In Vitro Biology, Tuson, USA, Jun. (2008)

6. Miharada K., Hiroyama T., Sudo K., Danjoh I., Nakamura Y.: "Lipocalin 2-mediated growth suppression is evident in human erythroid and monocyte/macrophage lineage cells." ISEH 37th Annual Scientific Meeting, Boston, USA, Jul. (2008)

7. Hiroyama T., Miharada K., Sudo K., Danjoh I., Nakamura Y.: "Establishment of mouse embryonic stem cell-derived erythroid cell lines able to ameliorate acute anemia." ISEH 37th Annual Scientific Meeting, Boston, USA, Jul. (2008)

8. Danjoh I., Sone H., Saijo K., Nagayoshi M., Iimura E., Kurita K., Nakamura Y.: "Collection of peripheral blood mononuclear cells obtained from amerindians and other mongoloid minority groups." XX International Congress of Genetics, Berlin, Germany, Jul. (2008)

【Domestic Conferences】 Total 51