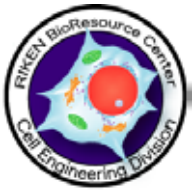
Cell Engineering Division



Head, Yukio NAKAMURA

Goal

Cell Engineering Division in RIKEN BioResource Center is a not-for-profit public bank that accepts donation and deposit of human and animal cell materials developed by life science research community. It examines, standardizes, amplifies, preserves, and provides cell materials to the interested scientists in all over the world. The major cell material has been cultured cell line, i.e., immortalized clone cells. Regarding human cell lines, the majority of them are derived from tumor cells. There is no doubt that the demand of supply of these cell lines will never cease in the field of biology. In addition, the demand for supply of non-immortalized cells has recently increased especially in the fields of developmental biology, transplantation medicine, regenerative medicine, and so on. To meet these demands, 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 conduct not only banking of cell materials but also research and development relating to cell materials, such as establishment of novel human and animal cell lines and development of new technology to utilize cell materials.

History

RIKEN Cell Bank was established in June 1987 when a committee of scientists recognized a 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 to Cell Engineering Division. In 2002, our division was recognized as the central archive for 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. Cell Banking: Human B cells transformed by EB virus
3. Cell Banking: Human mesenchymal stem cells
4. Cell Banking: Human umbilical cord blood cells
5. Quality control by STR profiling analysis for human cell lines
6. Ethical matter relating to human cell materials
7. Good manufacturing practice (GMP) facility

2003 ~ 2005

Activities relating to development of new cell resources

1. Development of technology to culture primate embryonic stem (ES) cells
2. Development of technology to obtain human feeder cells that maintain ES cells
3. Development of technology to establish human and animal cell lines possessing multi-potency
4. Development of technology to improve cell culture
5. Development of technology to immortalize human primary cells

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Activities relating to cell banking

1. Cell Banking: Human and animal cell lines

Cell lines are very useful materials in all fields of biology and they have been utilized in many kinds of experiment. We possess approximately two thousands cell lines, of which approximately one thousand lines are available for distribution. Approximately a half of the cell lines were derived from human cancer cells. These cancer tissue-derived cell lines are very precious 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, we possess several kinds of mouse embryonic stem (ES) cell line including C57BL/6-derived ES cell line. In recent years, nearly three thousands ampoules have been distributed in a year (Figure 1), mostly to not-for-profit 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 those cell lines in the field of biology will never cease.

2. Cell Banking: Human B cells transformed by EB virus

In order to analyze the causes of certain specific diseases at the level of genome, many genome samples are required. However, it is not so easy to collect many samples. Thus the collection of many genome samples and/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 volunteers possessing certain disease but also healthy volunteers.

3. Cell Banking: Human mesenchymal stem cells

Compared to obtaining primary cells derived from experimental animals, it is very difficult to obtain human primary cells. Current researches in life science, however, require human primary cells, such as stem cells, especially in the fields of transplantation and regenerative medicine. We succeeded to establish a system to provide such human primary cells efficiently. By collaborating with researchers who developed technologies to expand human mesenchymal stem cells *in vitro* very efficiently, we are supplying human mesenchymal stem cells to researchers. Mesenchymal stem cells can differentiate to bone, cartilage and reportedly also to neurons and cardiomyocytes (Figure 2).

4. Cell Banking: Human umbilical cord blood cells

Umbilical cord blood is a source of not only hematopoietic stem cells but also other somatic stem cells (Figure 3). Human umbilical cord blood cells are readily available, but usually discarded if they are not used in transplantation. Provided the mother of a newborn baby agrees to allow the umbilical cord blood cells to be used in research, the material can provide a valuable resource without the complicating factor of ethical concerns. By collaborating with "Japanese Cord Blood Bank Network", we are supplying human umbilical cord blood to domestic researchers in order to contribute for the fields of transplantation and regenerative medicine (Figure 4).

5. Quality control by STR profiling analysis for human cell lines

Cross-contamination between cultured cell lines can result in the generation of erroneous scientific data. Hence, it is very important to eliminate cell lines that are of an origin different from that being claimed. Inter-species 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 prior to the development of technology that detects differences between cell lines at the molecular level. Recently, profiling of short tandem repeat (STR) polymorphisms (STR profiling) has been established as a method for the analyses of gene polymorphism. STR profiling is a simple and reliable method to identify individual cell lines. All human cell lines we are currently providing were analyzed by STR profiling to authenticate its identity. We found that more than ten human cell lines out of approximately 400 were misidentified. We conclude that STR profiling is a useful and powerful method to eliminate cell lines that have been misidentified by cross-contamination or by other causes. Through this unfortunate lesson, we have established a method for rigorous quality control.

6. Ethical matter relating to human cell materials

Cell banking of human cells requires strict regulation about ethical matters. We only accept the donation of human cells that are approved by the Institutional Review Board (IRB) at RIKEN Tsukuba Institute. Furthermore, RIKEN BRC contracts Material Transfer Agreement (MTA) with the organization that donates human cells to RIKEN BRC. In the MTA, RIKEN BRC confirms that the human cell resource was obtained after a proper informed consent. An approval by the IRB in the organization that donates human cells to RIKEN BRC is also necessary. When RIKEN BRC distributes human cells to user, RIKEN BRC also contracts MTA with the user. As for certain human cells, such as umbilical cord blood, an approval by the IRB at the user organization is also required.

7. Good manufacturing practice (GMP) facility

Basically, we are always trying to improve our technology for cell banking, such as culturing method and preserving method. In order to maintain the quality of human cell resources, we established Good Manufacturing Practice (GMP) facility in 2002 (Figure 5). We are culturing human hematopoietic stem cells in umbilical cord blood and human mesenchymal stem cells in the facility.

Activities relating to development of new cell resources

1. Development of technology to culture primate embryonic stem (ES) cells

Embryonic stem cells have a lot of potential not only in the field of basic biology but also in the field of clinical science. Before human ES cells are applied to clinical science, many kinds of experiment using primate ES cells are required. Therefore, we are planning banking of primate ES cells. At the moment we are establishing the technology to culture primate ES cells using cynomolgus monkey ES cell line.

2. Development of technology to obtain human feeder cells that maintain ES cells

To our knowledge all human ES cell lines existing at the moment are cultured on the feeder layer derived from mouse embryo, i.e., Mouse Embryonic Fibroblast (MEF). Considering the application of human ES cells to clinical science, the cells cultured on MEF are not appropriate. Feeder cells derived from human resources are necessary and required, however the technology to obtain human feeder cells constantly is not established yet. We are planning to establish such technology using fibroblastic cells derived from placenta and umbilical cord.

3. Development of technology to establish human and animal cell lines possessing multi-potency

Human and animal cell lines possessing multi-potency and/or tissue-specific character are very useful for developmental biology and the basic research of regenerative medicine. We are trying to establish such cell lines by various approaches. First, identification and purification of tissue-specific stem cell may lead to establishment of such cell line by immortalizing the stem cell. Second, induction of ES cell's differentiation may lead to such cell line. Third, reprogramming of somatic cells may lead to such cell line. We are investigating these possibilities.

4. Development of technology to improve cell culture

All kinds of cell are affected by many humoral factors both *in vivo* and *in vitro*. Analyses of functions of these humoral factors are essential for improvement of cell culture and cell differentiation. Search for novel humoral factors is also one of the most important researches in this field.

5. Development of technology to immortalize human primary cells

It is very difficult to immortalize human primary cells. This fact makes the difficulties of using human cells in many fields of research. To immortalize human primary cells efficiently, we are analyzing mechanisms associating with cell mitosis and apoptosis.

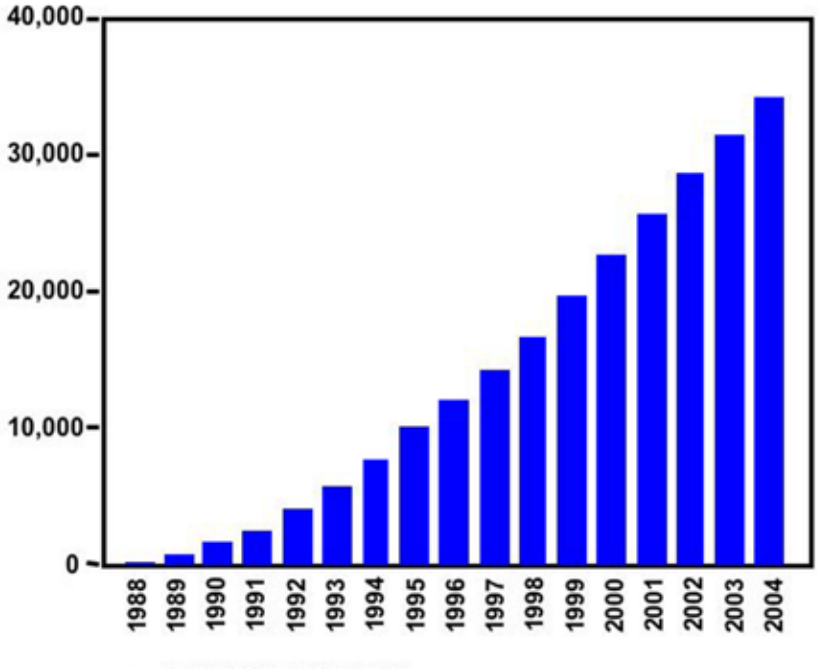

Figure 1.Accumulated numbers of provision of cell materials.

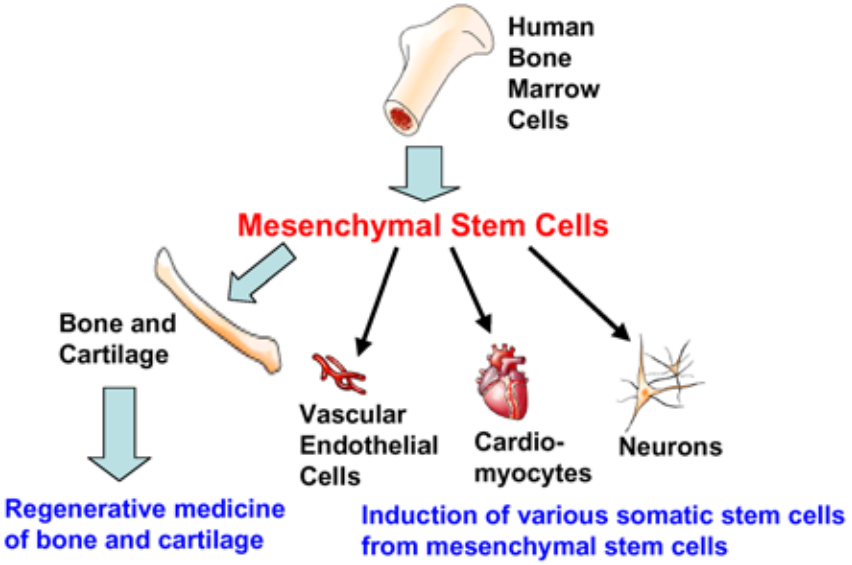

Figure 2. Usefulness of human mesenchymal stem cells. Human mesenchymal stem cells that are originally donated for regenerative medicine etc. and expanded in vivo will be supplied to researchers depending on the informed consent of patients.

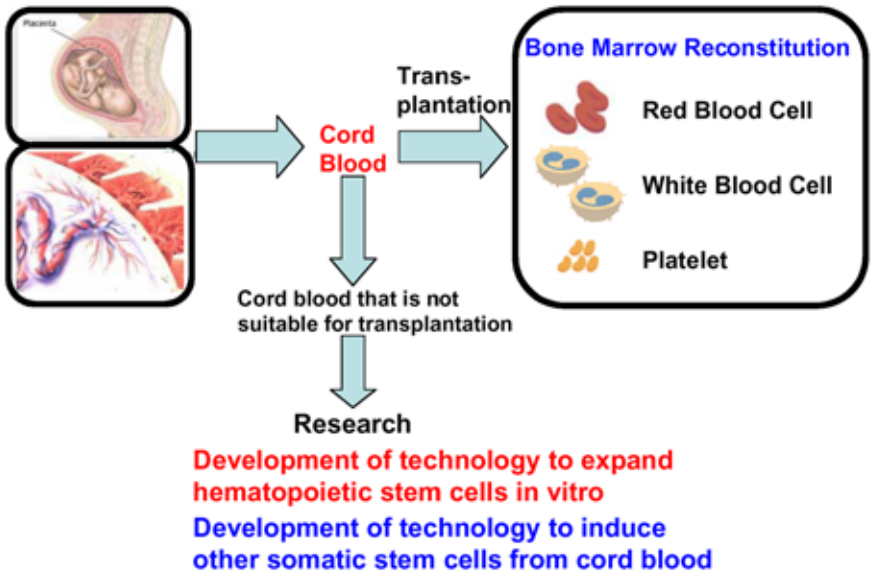

Figure 3. Flow chart of human umbilical cord blood samples.

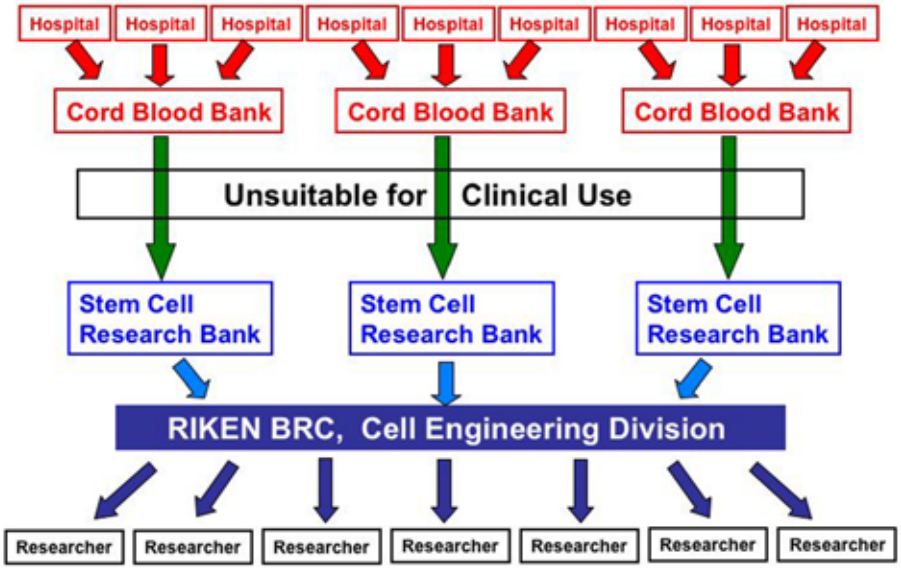

Figure 4. Scheme of banking of human cord blood. Human cord blood that is originally donated for transplantation but is not suitable for the purpose will be supplied to researchers depending on the informed consent of donators.

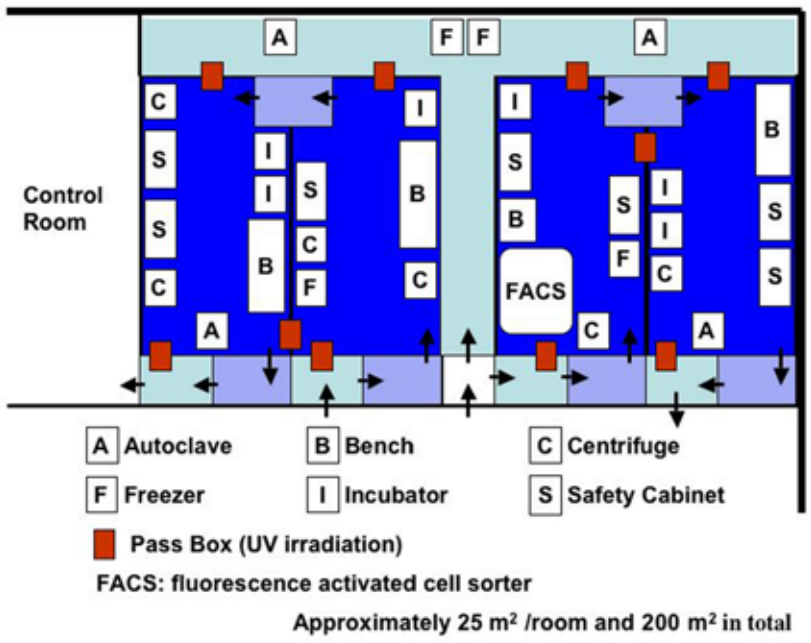

Figure 5. GMP (good manufacturing practice) facility.

Publications

Original Papers (* Peer reviewed Journal)

1. Tadao Ohno: Autologous cancer vaccine: A novel formulation. Microbiol. Immunol. 47: 255-263 (2003) *

2. Kaoru Saijo, Mayako Kato, Takashi Oomori, Isao Yoshimura, Tadao Ohno: The Validation study which leads organizers “cry”: So many points you must follow. AATEX 9: 54 (2003) *

3. Fabian Emura, Hiroshi Kamma, Mila Ghosh, Naoto Koike, Toru Kawamoto, Kaoru Saijo, Tadao Ohno, Nobuhiro Ohkouchi, and Takeshi Todoroki: Establishment and characterization of novel xenograft models of human biliary tract carcinomas. Int. J. Oncol. 23: 1293-1300 (2003) *

4. Lan Huang, Tadao Ohno: Protective anti-tumor immunity induced by fixed tumor cells in combination with adjuvant in a murine hepatoma model. Cancer Letters 202: 153-159 (2003) *

5. Oikawa T., Kawai K., Ishikawa I., Ohno T., and Akaza H.: Induction of potent anti-tumor natural-killer cells from peripheral blood of patients with advanced prostate cancer. Scientific Discovery 92: 1009-1015 (2003) *

6. Fujiki, Y., Fukawa, K., Kameyama, K., Kudo, O., Onodera, M., Nakamura, Y., Yagami, K-i., Shiina, Y., Hamada, H., Shibuya, A., and Nakauchi, H.: “Successful xenotransplantation of human hematopoietic progenitor cells into pig”, Transplantation 75: 916-922 (2003)*

7. Eiichi Ishikawa, Koji Tsuboi, Shingo Takano, Eiji Uchimura, Tadao Nose and Tadao Ohno:

Intratumoral injection of IL-2-activated NK cells enhances the antitumor effect of intradermally injected paraformaldehyde-fixed tumor vaccine in an rat intracranial brain tumor model. Cancer Sci. 95: 98-103 (2004) *

8. Ming Kuang, Bao Peng, Ming D. Lu, Li J. Liang, Jie F. Huang, Qiang He, Yun P. Hua, Saeri Totsuka, Shu Q. Liu, Kam W. Leong, and Tadao Ohno: Phase II Randomized Trial of Autologous Formalin-Fixed Tumor Vaccine for Post-surgical Recurrence of Hepatocellular Carcinoma. Clinical Cancer Research 10: 1574-1579 (2004) *

9. Hideki Harada, Satoru Watanabe, Kaoru Saijo, Isamu Ishiwata, and Tadao Ohno: A Wilms tumor cell line, HFWAT, can greatly stimulate proliferation of CD56+ human natural killer cells and their novel precursors in blood mononuclear cells. Experimental Hematology 32: 614-621 (2004) *

10. Mila Ghosh, Naoto Koike, Go Yanagimoto, Shin-ichi Tsunoda, Sumil Kaul, Takashi Hirano, Fabian Emura, Hironobu Kashiwagi, Toru Kawamoto, Nobihiro Ohkohchi, Kaoru Saijo, Tadao Ohno: Establishment and characterization of unique human gallbladder cancer cell lines. INTERNATIONAL JOURNAL OF ONCOLOGY 24: 1189-1196 (2004) *

11. Kushida, S., B.G.Peng, E. Uchimura, M. Kuang, L. Huang, M. Miwa, T. Ohno: A tumour vaccine of fixed tumour fragments in a controlled-release vehicle cytokines for therapy of hepatoma in mice. Digestive and Liver Disease 36: 478-485 (2004) *

12. Hideki Harada, Kaoru Saijo, Isamu Ishikawa, and Tadao Ohno: A GFP-Transfected HFWT Cell Line, CHINK-1, as a Novel Target for Non-RI Activated Natural Killer Cytotoxicity Assay. Human Cell 17: 43-48 (2004) *

13. Eiichi Ishikawa, Koji Tsuboi, Kaoru Saijo, Hideki Harada, Shingo Takano, Tadao Nose and Tadao Ohno: Autologous Natural Killer Cell Therapy for Human Recurrent Malignant Glioma. Anticancer Research 24: 1861-1872 (2004) *

14. Mila Ghosh, Naoto Koike, Shin-ichi Tsunoda, Takashi Hirano, Sunil Kaul, Hironobu Toru Kawamoto, Nobuhiro Ohkohchi, Kaoru Saijo, Tadao Ohno, Masanao Miwa and Takeshi Todoroki :Characterization and Genetic Analysis in the Newly Established Human Bile Duct Cancer Cell Lines. International Journal of Oncology 26: 449-456 (2004) *

15. Eiichi Ishikawa, Koji Tsuboi, Kaoru Saijo, Shingo Takano and Tadao Ohno : X-irradiation to Human Malignant Glioma Cells Enhances the Cytotoxicity of Autologous Killer Lymphocytes Under Specific Conditions. International Journal of Radiation Oncology Biology Physics 59(5): 1505-1512(2004) *

16. Koji Kawai, Kaoru Saijo, Takehiro Oikawa, Tadao Ohno and Hideyuki Akaza: Enhancement of T

Cell Proliferative Response Aginst Autologus Cancer Cells of a Metasatic Renal Cell Carcinoma Patient after Unexplained Regression. International Journal of Urology 11:1130-1132(2004)*

17. Zhongyan Zhang, Ken-ichi Hirano, Kosuke Tsukamoto, Chiaki Ikegami, Masahiro Koseki, Kaoru Saijo, Tadao Ohno, Naohiko Sakai, Hisatoyo Hiraoka, Ichiro Shimomura and Shizuya Yamashita : Defective cholesterol efflux in Werner syndrome fibroblasts and its phenotypic correction by Cdc42, a RhoGTPase. Experimental Gerontology 40 : 286-294(2005)*

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

21. Yoshino, K., Iimura, E., Saijo, K., Iwase, S., Fukami, K., Ohno, T., Obata, Y., and Nakamura, Y. Essential role for gene profiling analysis in the authentication of human cell lines. Human Cell (in press) *

22. Miharada, K., Hiroyama, T., Sudo, K., Nagasawa, T., and Nakamura, Y. Refinement of cytokine use in the in vitro expansion of erythroid cells. Human Cell (in press) *

Oral Presentations

1. Yukio Nakamura, Takashi Hiroyama, Mariko Nagayoshi, Kaoru Saijo, Kazuhiro Sudo, Emi Iimura, Kanae Kurita, Saeri Totsuka, Naoko Aoki, Yuichi Obata: "Human and Animal Cell Collection at RIKEN BioResource Center" 10th International Congress for Culture Collection (ICCC) (2004)

2. R. Kurita, E. Sasaki, T. Hiroyama, Y. Nakazaki, K. Izawa, H. Ishii, Y. Tanioka, K. Hanazawa, M. Osonoi, T. Hashiguchi, YS Bai, Y. Soda, S. Watanabe, S. Asano, K. Tani: "Hematopoietic cell differentiation of common marmoset (Callithrix jacchus) embryonic stem cells and their genetic manipulation using the third genration lentiviral vector" 7th Annual Meeting of the American Society of Gene Therapy (Minneapolis USA 2004)

3. Yukio NAKAMURA: "Banking of human and animal cells: National BioResource Project", The 26th Annual Meeting of The Molecular Biology Society of Japan, Kobe (2003)

4. Takashi HIROYAMA, Toyoki MAEDA, Yukoh NAKAJIMA, Ryo KURITA, Kazumi ODA, Rie ARIYOSHI, Kenichi MIHARADA, Tsuyoshi FUJIOKA, Rui MAEDA, Yukio NAKAMURA, Toshitaka MUTOH, Tatsuya KAWAGUCHI, Hideki NAKAKUMA, Taroh KINOSHITA, Kenzaburou TANI: "Suppression of megakaryocytic differentiation of GPI-anchored protein defficient K562 cells and recovery from the suppression by Musashi1.", The 26th Annual Meeting of The Molecular Biology Society of Japan, Kobe (2003)

5. Rui MAEDA, Kenichi MIHARADA, Takashi HIROYAMA, Tsuyoshi FUJIOKA, Kenichi YAGAMI, Yukio NAKAMURA: "Functional analysis of D type Cyclin in hematopoiesis", The 26th Annual Meeting of The Molecular Biology Society of Japan, Kobe (2003)

6. Kenichi MIHARADA, Mitsujiro OSAWA, Takashi HIROYAMA, Tsuyoshi FUJIOKA, Rui MAEDA, Toshiro NAGASAWA, Yukio NAKAMURA: "Role of 24p3, a novel transporter of iron, in hematopoiesis", The 26th Annual Meeting of The Molecular Biology Society of Japan, Kobe (2003)