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.

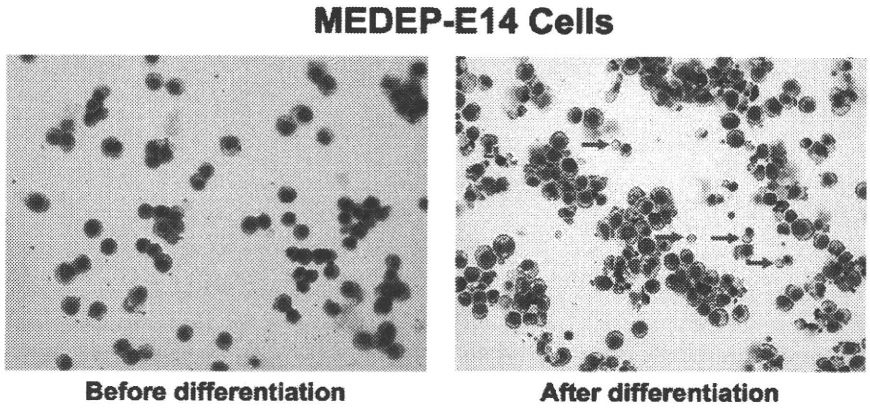

Fig. 4. An erythroid cell line established from mouse ES cells (E14), MEDEP-E14. After induction of differentiation, enucleated red blood cells can be detected. Red arrows indicate enucleated red blood cells.

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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E-mail address for questions regarding deposit and donation of cell lines 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 19813 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.

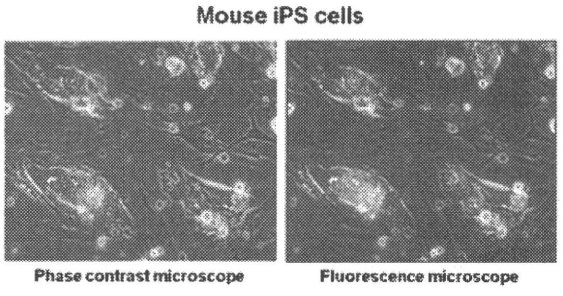
A search for alternative technologies to therapeutic cloning

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].

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.

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

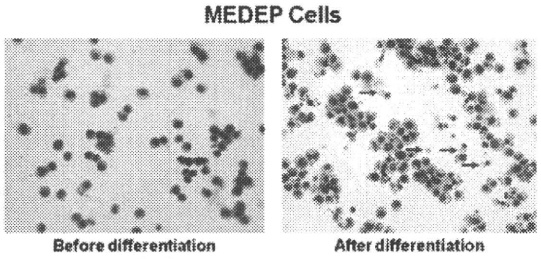
The ability to produce iPS cell lines will be benefit both regenerative medicine and also research into a variety of diseases[31-39]. For example, it is currently not possible to obtain neural cells from patients suffering from neural diseases. However, iPS cell lines could be established from their 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



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

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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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in the contents of the databases and in the services offered are being introduced.

Here, we outline some details of the BioResourceWorld (BRW) integrated database and several representative component databases. All the information described is available at http://www.nbrp.jp/.

NBRP Information Site is a gateway to access all the information of NBRP. The menu on the left-hand side beneath the heading 'Resource Center' provides links to all 30 individual databases, and gives the user direct access to the appropriate database for the organism of interest. The text box 'Keyword' near the upper center of the page is a query box for entering keyword searches of the BRW integrated database. Users can access the home page of BRW directly by clicking on 'Resource Integrated Search Site' in the menu bar near the top of the page. This menu bar also provides links to other pages: 'Journal' provides a link to the Research Resource Circulation (RRC) Web site for browsing and submission of papers reporting research using resources obtained through NBRP; 'Japan Genetic Resources' provides a link to a list of URLs for resources in Japan, but outside the NBRP group; and 'Worldwide Genetic Resources' provides a link to a list of URLs for bioresources worldwide. The total number of records currently available in the database for all bioresources and the subtotals for three individual classifications (animals, plants and microbes) are shown in the turquoise-colored box near the top left of the page.

NBRP DATABASES

An integrated NBRP database retrieval system: BioResourceWorld

BRW is an integrated database retrieval system that allows users to retrieve resources by using the body of information held by NBRP on a number of organisms. All the resources (~4.5 million items) are available for distribution. Keyword searching of BRW is directly available from the NBRP home page. A user performing a search can specify an organism and a resource category from pull-down menus. (The default values are 'All organisms' and 'All categories'.) When a user enters keywords and performs a search with the default settings, the total number of results is shown together with a breakdown of the number of strains and the number of DNA clones. This is followed by the list of resources broken down by organism. By clicking on a desired resource on the list, the user can access a detailed information page that provides links to the distribution-request site for each database (where this exists).

The BRW home page, which is linked from the top menu bar of the NBRP home page, contains the search functions described above and four tabs (ALL, DNA, BLAST and Gene Ontology). These four tabs provide, respectively, a summary display of all resources by organism groups, a summary display of DNA clones by organism groups, a BLAST search and a Gene Ontology (GO) search.

The BLAST search allows the user to search all organism groups and also to specify particular organisms or categories, such as genomic clones, cDNA clones, or libraries. The search results are shown as diagrams of alignments in which abbreviations of names of organisms or groups of organisms appear in a color-coded manner on the left-hand side of the diagram to help users recognize particular organisms. Individual sequences in hits have links to NCBI (1), DDBJ (2) and BRW, which gives the user access to the web sites from which the appropriate resources can be ordered.

The latest service of BRW is a search of resources on the basis of GO. The user can examine the hierarchical structure of a GO term and query it by using GO ID, GO term, or GO Gene. For example, if a user enters '0000038' (or 'very-long-chain fatty acid metabolic process') into the GO-ID (or GO Term), a resource list containing 'mouse (5/5), drosophila (2/4), arabidopsis (10/10)' is shown. The numbers in parenthesis indicate the number of resources associated with this GO-ID (left) and its descendants (right). Thus this service allows users a more semantic search and provides them with a wider range of resources.

The GO search is currently at a testing stage, because not all resources contain GO information. The total number of resources mapped to a GO Term is displayed, and a list of relevant resources can be obtained by clicking the figures. Like other search modes, the GO allows the ordering of resources from search results. We plan to enhance the search of resources across organism by the addition of supporting bio-ontology data, such as phenotype, anatomy and development.

The information contained in BRW is updated concurrently with that in the individual databases.

Resource research circulation

If a researcher obtains good results with NBRP resources, such a result may be useful for later researchers who use the same resources. We therefore ask researchers to feed back information on papers that report the results of their studies, and we also collect other papers in which NBRP resources are used. This information is used to create an open database (RRC) of papers related to the NBRP resources. RRC also provides an online registration system through which a paper can be submitted merely by entering its PubMed identification number and the name of the resource used in the paper. We have asked many researchers to use this system to feed back on the papers that they have published. It would be ideal for experimental researchers if, as in the case of the accession numbers of the DNA Data Bank, we could establish a system in which detailed information on the bioresources described in 'Materials and Methods' sections of papers could be obtained from public databases and, furthermore, the materials themselves could be easily acquired.

The NBRP databases

Table 1 shows the names and features of the NBRP component databases, organized by organism group. As shown in Table 1, the NBRP resource collection covers

a wide range of taxa with indications of the presence or absence of the following collections: (i) naturally mated lines (including wild species, cultivated species, inbred lines and spontaneous mutants); (ii) genetically engineered organisms (including induced mutants, transgenic strains, transposon-inserted strains, deletion strains, consomic lines, genome-wide knockout strains, and enhancer trap lines); and (iii) DNA (plasmids, vectors and genomic/cDNA clones). The numbers of records of resources vary according to the group of organisms, and the resources in classification (3) account for 95% of the total. The External DB column in Table 1 indicates databases that have one-way links or cross links to external comprehensive databases for the model organisms. The Collaborating DB inside the parenthesis indicates an external database with which the NBRP database collaborates.

Types and names (identifiers) of the resources, and distribution/deposition methods (such as MTA) are essential information for all resources. For DNA clones, the accession numbers in the DNA Data Bank, together with homologous sequences, are the items of information common to all organism groups. NBRP also includes some activities in which the distribution of resources requires a prescribed review procedure (such as the Human ES-cells and Macaque). Activities where resources cannot be obtained by proliferation, but require non-invasive sampling or sampling from dead animals, are provided only for research that meets prescribed standards [such as the Great Ape Information Network (GAIN)]. The Global Biodiversity Information Facility (GBIF; http://www.gbif.org/) is an activity that forms the Japanese node of an international project that is involved in collecting and creating a database on specimens and observation data held in museums. Information from the GBIF and resources from the GAIN and Macaque databases are generally not distributed, so that they are not included in BRW.

As illustrative examples of the features described above, the databases for rice, *Escherichia coli*, *Caenorhabditi elegans* and the rat are described in more detail below, and some details of the RIKEN BioResource Center databases are also given.

NBRP-rice (Oryzabase) (25). Oryzabase, a comprehensive database that is currently used by many researchers, succeeded the Database of Resources and Trait Genes, which was launched in 1995. Genomic information has been added to the database on resources and genes so that it has become comprehensive and now functions as the NBRP database. The characteristic wild-rice collection consists of three different core collections. Core collection Rank 1 relates to 18 species from nine genomes and contains detailed phenotype data and many photographs. The collection of unique mutant strains includes strains for which trait genes have been identified, as well as strains that have phenotypes for which the responsible genes are not known. The former set of strains can be retrieved on the basis of genetic information, and the latter set of strains can be retrieved on the basis of the phenotype. The data on both sets of strains include photographs.

We constantly update the dictionary of trait genes with data extracted from published papers, and we have also been promoting the establishment of correspondences between trait genes and the accession numbers of DNA sequences, ORF numbers of genome projects and displays of chromosomes by using physical maps. Oryzabase also provides access to a Web site for online submission of new rice genes, which is based on the Gene Nomenclature System for Rice as determined by the Committee on Gene Symbolization, Nomenclature and Linkage of the Rice Genetics Cooperative (CGSNL) (26). Users can give feedback on an individual gene through the detailed pages for that gene.

Basic information on rice, including the definitions of the tissue-specific developmental stages (such as embryo, inflorescence, leaf, root, anther, ovule, pollen mother cell, stoma or vascular bundle) and information on tissue-specific or developmental stage-specific gene expression are also available through this web site.

A version of Textpresso, a text-mining system for scientific papers developed by the Generic Model Organism Database and adapted for use with rice, has been constructed and is available through Oryzabase. It provides access to a total of ~20 000 rice-related papers (abstracts and titles) in PubMed, and is linked from the home page of Textpresso.

Oryzabase has links to two external databases: Gramene and IRRI. Genetic information is linked to the former database, and wild-strain information is linked to the latter. Oryzabase also allows BLAST searches to be made through all genomes or by chromosomes, provides a tool for extracting the specified region of genome sequences, and can provide downloadable text files of almost all information.

NBRP-E. coli. Of all the organisms in the NBRP collection, *E. coli* was the first to have its genome sequence determined. A feature of NBRP-*E. coli* is its genome-wide genetically engineered strain collections. At the web site of NBRP-*E. coli* (http://www.shigen.nig.ac.jp/ecoli/strain/), users can browse outlines and lists of collections and search full data or perform queries by specifying details. Although online requests are available, these often take time because of the complexity of the MTA process. For the convenience of users, a tracking system has been introduced that allows users to check the progress of their requests in real time.

The information center maintains profiling of *E. coli* chromosomes (PEC), an information site of a project on essential *E. coli* genes (27). Through PEC, the information center has made genome maps and genetic information available. The resource information contained in NBRP-*E. coli* is cross linked with PEC to permit access to resources through the maps. For example, by clicking the deletion regions of extensive deletion mutants, or by clicking the gene parts of mutants mutated by gene units, the user can access detailed pages that are linked to request pages for the resources. PEC has many links to external databases, including NCBI, UniProtKB, COG,

Table 1. The NBRP databases

	Database name	Taxon	Wild/ inbred/ landrace	Mutants	Plasmid/ vector/ clones	Map (Physical or Linkage)	Gene (mutated genes/all genes)	Phenotype	SNPs	Blast service	Images	External DB (collaborating DB)[reference]	Featured contents/ services
1	NBRP-Macaque	Vertebrata-mammalia-primate	o		o								
2	NBRP-Mouse (Riken BRC)	Vertebrata-mammalia-murinae-mus	o	o	o	P	M	o	o	o	o	MGI[3], (IMSR, JMSR;http://www.shigen.nig.ac.jp/mouse/jmsr/)	
3	NBRP-Rat	Mammalia-murinae-rattus	o	o	o	P	M	o	o	o	o	RGD[4], (JMSR)	graphical display of the phenotype data
4	NBRP-Xenopus	Vertebrata-amphibia	o		o					o			
5	NBRP-Zebrafish	Vertebrata-actinopterygii-cypriniformes-danio		o			M	o			o	ZFIN[5]	
6	NBRP-Medaka	Vertebrata-actinopterygii-beloniformes-oryzias	o	o	o	P	M	o		o	o		atlas, phylogenetic tree
7	NBRP-Ciona	Ascidiacea-ciona		o	o		M	o			o	(Ghost,[6] CIPRO;http://cipro.ibio.jp/new/)	
8	NBRP-Drosophila (DGRC)	Arthropoda-insecta-diptera-drosophilidae	o	o	o	P	A	o	o	o	o	FlyBase[7]	
9	NBRP-Silkworm (SilkwormBase)	Arthropoda-insecta-lepidoptera-bombyx		o		L	M	o			o		laeva period time
10	NBRP-*C. elegans*	Pseudocoelomata-nematoda		o			M					Wormbase[8]	
11	NBRP-Rice (Oryzabase)	Viridiplantae-poaceae-oryzeae	o	o		P,L	A	o		o	o	Gramene[9], IRRI; http://beta.irri.org/index.php/Home/Welcome/Frontpage.html	developmental stage
12	NBRP-Barley	Viridiplantae-poaceae-triticeae-hordeum	o		o	L		o		o	o		phenotype data
13	NBRP-Wheat (KOMUGI)	Viridiplantae-poaceae-triticeae	o	o	o	L	A	o		o	o	(TriFLDB[10])	gene catalogue
14	NBRP-*Arabidopsis* (Riken BRC)	Viridiplantae-brassicales-arabidopsis		o	o			o		o	o		
15	NBRP-*Chrysanthemum*	Viridiplantae-asterids-chrysanthemum	o					o			o		
16	NBRP-Morning glory	Viridiplantae-solanales-ipomoeeae		o	o	L	M	o			o		
17	NBRP-Lotus/ Glycine (LegumeBase)	Fabales-fabaceae	o	o	o	L	M	o			o	(miyakogusa.jp[11], Soybean Full-length cDNA database[12])	
18	NBRP-Tomato	Viridiplantae-solanales-lycopersicon			o							(KafTom;http://www.pgb.kazusa.or.jp/kaftom/, MiBASE[13])	
19	NBRP-Algae	14 phyla (eukaryota and bacteria)	o					o			o		photograph, phylogenetic tree
20	NBRP-Yeast	Fungi-ascomycota		o	o	P	A					SGD[14], geneDB[15]	
21	NBRP-Cellular slime mold	Mycetozoa-dictyostelliida			o							dictyBase[16]	
22	NBRP-Prokaryote *E. coli*	Bacteria-proteobacteria		o	o	P	A			o		PEC[17], EcoGene[18], EcoCyc[19], COG[20], NCBI[1], SwissProt[21], GTOP[22], KEGG[23], InterPro[24]	
23	NBRP-Prokaryote *B. subtilis*	Bacteria-firmicutes-bacilli-bacillaceae		o			M						
24	NBRP-Pathogenic microbes	Bacteria and protozoa	o					o			o		
25	NBRP-General microbes (Riken BRC, JCM)	Bacteria	o										

EcoCyc, GTOP, EcoGene and KEGG. We also perform similarity searches on gene sequences to add domain information from Pfam and PROSITE. In addition, PEC provides BLAST search through two different strains, MG1655 and W3110; all ORF, and essential genes. The tool for specifying the desired fragments of genome sequences is also available. Almost all the information in the database can be downloaded in the form of text files.

NBRP-C. elegans. NBRP-*C. elegans* (http://www.shigen.nig.ac.jp/c.elegans/) is a smaller database than the two databases discussed above. Each record consists only of the allele of the deletion strain, a systematic identification tag for the gene (the CGC name), information on the position of the gene on the chromosome, information on the positions of deletion regions, and primer information. However, the CGC name, allele and sequence are cross linked to the corresponding page of WormBase, a comprehensive database on *C. elegans*, providing an easy access to that database. In the case of this resource, requested mutants are isolated from the pool after the request. Users can check on the state of progress of isolation online. Mutants that have been isolated once will be listed as available mutants (isolated), so that other users can request the same mutants. NBRP-*C. elegans* is unique in that information on researchers who have received mutants from this project is made public online. Because almost all papers in which the resources are used contain the names of these resources, and the information is fed back from paper-registration sites, the *C. elegans* database automatically reflects information in the RRC. This is a model case of good circulation between resources and researches.

NBRP-rat. Because the database of rat resources (http://www.anim.med.kyoto-u.ac.jp/NBR/) contains substantial characterization data from individual resources and has many tools for browsing this data, it is efficient in allowing researchers to find the resources best suited to their research from a range of trait information. For example, the top page displays a pie chart that shows a breakdown of the research fields in which the resources are used, and by clicking a research field of interest, the user can obtain a list of resources related to that particular research field. Another example, 'Phenome Project' provides 109 items of physiological, behavioral and anatomical phenome data in nine tables and in strain-distribution maps with the two items selected by the user as the abscissa and ordinate axes. Users can access detailed pages for strains by clicking data points in the maps. 'Genome' provides access to polymorphism data for 357 simple sequence-length polymorphism (SSLP) markers, obtained from investigations on more than 150 strains. A phylogenetic tree, constructed from polymorphism data, is also available, through which users can access detailed pages on resources.

Besides characterization data, the database also contains detailed pages on resources, including the preservation status, genetic status, research category, origin, genotype, references and a link to the Rat Genome Database (RGD). Recent enhancements include a BAC browser for F344 and LE BAC end sequences, and the addition of functional polymorphism data obtained by comparing 16 disease-associated gene mutations among multiple strains. The ENU-induced mutant archive (28) is also provided at this site.

NBRP-RIKEN BRC

The RIKEN BioResource Center (BRC) was established in 2001 with the aim of becoming the finest core bioresource facility in the world. Since then, it has been engaged in collecting bioresources developed mainly by Japanese scientists. These bioresources include living strains of mice and *Arabidopsis*, human and animal cells, DNA materials and various microbes for which the RIKEN BRC has been designated the national core facility by the NBRP. The RIKEN BRC preserves these bioresources under conditions of strict quality control for provision to the scientific community. The RIKEN BRC also collects information on the whereabouts and characteristics of the bioresources, constructs databases, and offers the bioresource information to the research community within and outside Japan.

The Animal Search System (http://www2.brc.riken.jp/lab/animal/search.php) allows keyword searches to be performed on >2000 strains of mice available from the RIKEN BRC, including transgenic, knockout, inbred, wild-derived, ENU mutant and congenic strains. The system also provides detailed information on each strain, such as the strain name, description, gene details, references, availability status, health report, depositor, specific terms and conditions for distribution, and image(s), which capture the characteristics of the strain. Some gene symbols have links to the Mouse Genome Informatics (MGI) database, which leads to further detailed information on the particular gene. As well as being publicly available, the up-to-date information on mice is also sent to the International Mouse Strain Resource (IMSR, http://www.findmice.org/) database, to which the RIKEN BRC is a contributing repository. The RIKEN BRC Mouse Phenome Database (RMPD) (http://www.brc.riken.jp/rmpd/mouse_phenome_top.html) contains phenotypic data on the physiology, biochemistry, hematology and morphology of inbred, mutant, wild-derived and recombinant inbred strains of mice, and enables biomedical researchers to find appropriate strains for their researches.

The *Arabidopsis* transposon tagged lines can be searched in a web-based catalogue (http://www.brc.riken.jp/lab/epd/catalog/transposon.html), where information on insert positions of transposons and adjacent genes can be obtained for more than 15 000 lines. The SENDAI *Arabidopsis* Seed Stock Center (SASSC) database (http://www.brc.riken.jp/lab/epd/SASSC/) provides information on its collection (wild type and mutant), such as strain name, region of collection and phenotypic remarks. The RIKEN *Arabidopsis* full-length cDNA (RAFL) clone database (http://www.brc.riken.jp/lab/epd/catalog/cdnaclone.html) contains more than 250 000 clones and the users can retrieve clones by

NCBI accession number, AGI code, clone name or sequence homology. The Systematic Consolidation of *Arabidopsis* and other Botanical REsources (SABRE) database (http://saber.epd.brc.riken.jp/sabre/SABRE0101.cgi) offers searches of BRC plant DNA resources across the species that it contains.

The database of human and animal cells (http://www2.brc.riken.jp/lab/cell/search.php) has its origins in the database of the RIKEN Cell Bank, which began collecting and distributing cell resources in 1987. Starting from a stand-alone database for internal use, it has been transformed to allow searching of cell resources and the provision of the associated information through the Internet. Because the Cell Bank began its activity as a division of the BRC, its stock list has grown with the addition of various new kinds of cell resources, such as Epstein–Barr virus-transformed B cell lines, human somatic stem cells, embryonic stem cell lines and induced pluripotent stem cell lines. The function of the database has accordingly been enhanced to allow the presentation of other items of information that differ from resource to resource. The information available therefore depends on the resource; for example, information on the origin, morphology, culture conditions, restrictions on distribution, results of short tandem repeat analyses, images, etc. can be obtained for a conventional cell line resources.

Before the BRC was established, DNA materials were collected and distributed as an activity of the RIKEN DNA Bank. A keyword search system (http://www.brc.riken.jp/lab/dna/search/index.html) is available for DNA clones, vectors and recombinant adenoviruses. Resources are retrieved by plasmid name, by gene name or symbol, or by accession number. In the Geneset Bank database (http://www.brc.riken.jp/lab/dna/en/GENESETBANK/index.html), more than 20 illustrations for principal gene pathways are implemented, and DNA materials can be easily found according to the gene pathways to which they belong.

The microbial resource collection in RIKEN was founded in 1980 as the Japan Collection of Microorganisms (JCM), and the construction of a database for the collection began at its inception. The Web-based online catalog database (http://www.jcm.riken.jp/JCM/catalogue.shtml) was launched in 1995, and it has since been improved and updated. It now provides access to information on more than 11 000 available strains, which are searchable by their accession number, scientific name and keywords on strain data. It is also possible to search for JCM strains that are equivalent to those in other culture collections. The database contains various useful items of information about strains, such as culture media and conditions, history (including isolation source), taxonomic data and references. The DNA sequence data, critical for the phylogeny and the genome data are linked to the DDBJ database for each strain.

Future directions

We will continue to improve the content of individual databases and upgrade the functions available at the integrated database-retrieval site. We also hope to expand external access to the databases and expand collaboration with other databases to permit access to our resources by a wider range of users. In particular, we will examine a new possibility for interconnecting reference data and resource databases with the aim of construction a virtual international network.

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Gene expression profiles of cryopreserved CD34$^+$ human umbilical cord blood cells are related to their bone marrow reconstitution abilities in mouse xenografts

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ABSTRACT

Human umbilical cord blood (UCB) cells are an alternative source of hematopoietic stem cells for treatment of leukemia and other diseases. It is very difficult to assess the quality of UCB cells in the clinical situation. Here, we sought to assess the quality of UCB cells by transplantation to immunodeficient mice. Cryopreserved CD34$^+$ UCB cells from twelve different human donors were transplanted into sublethally irradiated NOD/shi-scid Jic mice. In parallel, the gene expression profiles of the UCB cells were determined from oligonucleotide microarrays. UCB cells from three donors failed to establish an engraftment in the host mice, while the other nine succeeded to various extents. Gene expression profiling indicated that 71 genes, including *HOXB4*, *C/EBP-β*, and *ETS2*, were specifically overexpressed and 23 genes were suppressed more than 2-fold in the successful UCB cells compared to those that failed. Functional annotation revealed that cell growth and cell cycle regulators were more abundant in the successful UCB cells. Our results suggest that hematopoietic ability may vary among cryopreserved UCB cells and that this ability can be distinguished by profiling expression of certain sets of genes.

1. Introduction

Human UCBs are an important source of hematopoietic stem cells for treatment of various hematological disorders [1] and malignant diseases [2]. UCBs have the advantages of a more relaxed histocompatibility requirement than bone marrow cells and of the absence of any risk to the donor in their collection. These advantages make UCBs a versatile option for bone marrow reconstitution therapy [3]. The clinical outcome of UCB transplantation is comparable to allogeneic bone-marrow transplantation [4,5].

However, it has been found that 10–20% of patients experience insufficient engraftment of UCB donor cells and that some cases may have no evidence of bone marrow rescue [4–8]. Success of UCB transplantation is associated with various factors, such as use of low-dose total-body irradiation, patient age, and less severe disease progression [5,9]. In addition to patient-related factors, it is also possible that the quality of the stem cells in the UCB, such as cell viability, pluripotency, and resistance to stress, affects the outcome of the allograft. Currently, the only available information on the influence of the quality of UCBs and outcome of the allograft is that a high dose of CD34$^+$ cells is required to ensure successful engraftment of transplanted UCBs [5,8,10]. One of the particular difficulties for assessing the influence of the quality of the UCBs on the outcome of therapeutic use is that the clinical condition of the recipients is so variable as to preclude any direct comparison of the quality of UCB allografts. Several factors, such as ethnicity, birth weight, sex, and type of delivery, are correlated with the ability of cryopreserved human UCBs to form colonies in *in vitro* culture [11].

Stevens et al. reported that the numbers of nucleated red blood cells are correlated with those of hematopoietic progenitor cells in human UCBs; moreover, UCBs with a high count of nucleated red blood cells show faster engraftment [12]. To date, however, no molecular indicators have been identified for assessing the quality of human UCBs. In this study, we used a bone marrow reconstitution assay to assess xenograft success in mice, and thus enable evaluation of the quality of human cryopreserved UCBs. Additionally, we performed gene expression profiling of these UCBs in order to identify possible molecular markers for quality assessment. For the former assay, we used sublethally irradiated immunodeficient NOD/shi-scid Jic (NOD/SCID) mice as they offer reproducible recipient conditions and are therefore ideal for investigating potential

Abbreviations: UCB, umbilical cord blood; NOD/SCID, non-obese diabetes/severe combined immune-deficient.

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