nies indicated that the isolate was a *Mycoplasma* species. The colony size corresponded to measures previously reported for the LC type (Thigpen et al., 1983). Furthermore, the ultrastructural characteristics of the ibex isolate, evaluated by electron microscopy, showed typical architecture of *Mollicutes*. Fine granulations were evident on the cell surface, corresponding to a "slime layer" made of galactan, known to be present on the surface of *Mycoplasma mycoides* cluster cells (Gourlay and Thrower, 1968). Despite these indications that the IKC was caused by a *Mycoplasma* species further work was required to identify it to subspecies level. This mycoplasma isolate was identified in part by PCR and PCR/DGGE methods, but the final identification was only confirmed by DNA sequencing of the 16S rDNA gene and phylogenetic analysis.

The difficulty of isolating *Mycoplasma* species rather than using molecular tests may have limited the diagnosis of IKC caused by *Mycoplasma* species in this study. However, based on other reports (Tschopp et al., 2005; Marco et al., 2009), a monitoring program may have considered only using a *M. conjunctivae* PCR, which would have also missed this diagnosis.

Considering that the *MmcLC* serovar can infect domestic and wild animal species, it is possible that wild animals may be a natural reservoir of infection potentially capable of transferring this pathogen to susceptible domestic animals and vice versa. Therefore control programs should address both domestic and wild animal populations simultaneously. This implies that monitoring of potentially susceptible wild animal populations and domestic animals farmed in areas close to habitats of wild fauna and application of measures to prevent interspecies transmissions are required.

Given the valuable domestic animal stocks farmed in the Valle d'Aosta Region, such as the 2,000 sheep and 5,000 goats (Istituto Zooprofilattico Sperimentale del Piemonte, 2008), the potential risk of contamination with a recognized causative agent of infectious agalactia, with subsequent restrictions with respect to current legislative provisions, has to be considered. According to the World Organisation for Animal Health (OIE), the *MmcLC* serovar is included in the list of diseases of importance to international trade (OIE, 2010). In addition, according to European Commission directives (European Commission, Directorate General for Health and Consumers, 1991) on animal health conditions governing intracommunity trade in ovine and caprine animals, adopted in Italy under Presidential Decree (Italian Republic, 1992), the pathogen is listed among other major infectious diseases of small ruminants, such as brucellosis, rabies, and anthrax, all of which require certification of clinical absence for fattening and breeding animals subject to trade.

Our study demonstrated that infection with *Mmc*LC serovar occurs in wild fauna in the northern Italian region of Valle d'Aosta and may be associated with clinical keratoconjunctivitis. This observation provides a novel facet of the study on the *Mycoplasma* spp. infection in the wild fauna of the Alpine regions of northern Italy, considering that several outbreaks of IKC have been reported, particularly in chamois and ibex, evidencing the circulation of *M. conjunctivae* (Grattarola et al., 1999; Belloy et al., 2003). This indicates the need for regular and intensive laboratory investigations for the differential diagnosis of clinical cases in wild ruminants suffering from ocular lesions, which are not always ascribable to *M. conjunctivae*. In the absence of specific serologic tests the use of molecular techniques such as PCR/DGGE on clinical specimens may be beneficial in determining the true incidence of infectious conjunctivitis caused by *Mycoplasma* species. The processes and expense of specific *Mycoplasma* diagnosis may contribute to underestimation of *Mycoplasma* infection prevalence in both

domestic and wild animals, especially where epidemiologic investigations are difficult.

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RESEARCH ARTICLE

Use of BAC array CGH for evaluation of chromosomal stability of clinically used human mesenchymal stem cells and of cancer cell lines

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Abstract Array-based comparative genomic hybridization (aCGH) using bacterial artificial chromosomes (BAC) is a powerful method to analyze DNA copy number aberrations of the entire human genome. In fact, CGH and aCGH have revealed various DNA copy number aberrations in numerous cancer cells and cancer cell lines examined so far. In this report, BAC aCGH was applied to evaluate the stability or instability of cell lines. Established cell lines have greatly contributed to advancements in not only biology but also medical science. However, cell lines have serious problems, such as alteration of biological properties during long-term cultivation. Firstly, we investigated two cancer cell lines, HeLa and Caco-2. HeLa cells, established from a cervical cancer, showed significantly increased DNA copy number alterations with passage time. Caco-2 cells, established from a colon cancer, showed no remarkable differences under various culture conditions. These results indicate that BAC aCGH can be used for the evaluation and validation of genomic stability of cultured cells. Secondly, BAC aCGH was applied to evaluate and validate the genomic stabilities of three patient's mesenchymal stem cells (MSCs), which were already used for their treatments. These three MSCs showed no significant differences in DNA copy number aberrations over their entire chromosomal regions. Therefore, BAC aCGH is highly recommended for use for a quality check of various cells before using them for any kind of biological investigation or clinical application.

Keywords Validation of cell line · BAC · BAC array CGH

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Introduction

Comparative genomic hybridization (CGH) and array-based CGH (aCGH) can detect DNA copy number aberrations in the entire human genome [1, 2]. In fact, to detect DNA copy number aberrations, aCGH has been used to examine many cancers and cancer cell lines for diagnosis and prognosis [3–7]. Moreover, in Korea an aCGH chip was approved for use to diagnose hereditary diseases and inherent chromosomal disorders, such as Down syndrome and Turner's syndrome, which are caused by chromosomal aberrations [Korean Food and Drug Administration (KFDA; http://www.macrogen.com/eng/macrogen/press_list.jsp)]. Bacterial artificial chromosome (BAC) aCGH has attracted attention as a superior method for genome-wide analysis not only to detect DNA copy number aberrations, but also to evaluate hereditary chromosomal disorders.

In recent years, regenerative medicine using mesenchymal stem cells (MSCs) has received much attention

[8, 9]. However, safety issues concerning the MSC applications, especially with respect to tumorigenesis, remain to be solved [9–11]. The BAC aCGH method would be useful for the evaluation of chromosomal stability and instability, which are closely related to tumorigenesis.

In this study, we performed BAC aCGH to evaluate chromosomal stability of HeLa cells, Caco-2 cells, and MSCs. The HeLa cell line was established as the first human cancer cell line derived from a cervical cancer and is one of the most widely used cell lines in the world [12, 13]. However, numerous other established cell lines are now used as a substitute for HeLa cells [13]. The Caco-2 cell line was established from a human colon cancer [14]. Even though the Caco-2 cell was derived from a colon cancer, it has been available for use as a convenient reference model for theoretical predictions of intestinal drug absorption in drug discovery [15]. Therefore, the stability of Caco-2 cells should be established for such a screening purpose. MSCs are expected to be applied for regenerative medicine, and they are already used clinically for the treatment of various diseases [16, 17]. The safety issue regarding the chromosomal stability of these cells thus becomes increasingly important for future clinical applications.

Materials and methods

Cell lines and DNA extraction

HeLa cells (human cervical cancer cell line) of three different numbers of passage times were used for this study. HeLa-A was purchased from the American Type Culture Collection (ATCC, Manassas, VA), and DNA was directly extracted without cultivation. The number of passage times of HeLa-A was approximately 100 according to an attached product information sheet from ATCC. HeLa-B and HeLa-C were obtained from the Japanese Collection of Research Bioresources (JCRB, Osaka, Japan), and the number of their passage times was 122 for HeLa-B and 150 for HeLa-C.

Three different types of Caco-2 cells (human colon cancer cell line) were also used for this study. Briefly, Caco-2 was purchased from ATCC and designated as Caco-2-a, and its DNA was directly extracted without any cultivation because this DNA was regarded as a control. Caco-2-b was maintained by a commercial institution and analyzed after 63 passage times. Caco-2-c was maintained by the same commercial institution, cultured on microporous membranes (0.4 μm diameter), and analyzed after 58 passage times.

The ACBRI-519 cell line, which was derived from normal human intestinal epithelial cells, was used as a counterpart of Caco-2 cells in this study. ACBRI-519 cells were purchased from Cell System Corp. (Kirkland, WA).

Three MSCs were derived from individual bone marrow samples that were actually used clinically [16]. MSC-1 was derived from a 69-year-old female and analyzed at passage number 3 after primary culture using bone marrow. In a similar manner, MSC-2 was derived from a 16-year-old female and analyzed at passage number 3. MSC-3 was derived from a 34-year-old male and analyzed at passage number 4. MSC-4 was derived from the same individual as MSC-3, but analyzed at passage number 7.

Extraction of genomic DNA was carried out by using SepaGene (Sanko Junyaku, Tokyo, Japan) except MSCs, and genomic DNA of MSCs was extracted by using the Gentra Puregene Cell Kit (Qiagen, Hilden, Germany). Each procedure of DNA extraction was according to the manufacturer's respective protocols.

BAC aCGH

BAC aCGH analysis was carried out as described previously [7]. Briefly, 500 ng of genomic DNA from a given cell line as the test sample and 500 ng of gender-matched reference genomic DNA (Promega Corporation, Madison, WI) were labeled with cyanine3-dCTP (Perkin Elmer Inc., Waltham, MA) for reference DNA or cyanine5-dCTP (Perkin Elmer) for test DNA by random priming in 50-μl reaction volumes by using the Bioprime DNA Labeling System (Life Technologies Corporation, Carlsbad, CA) and Array Kit (Macrogen, Seoul, Korea, http://www.macrogen.com). After labeling, unincorporated fluorescent nucleotides were removed by using a QIAquick polymerase chain reaction (PCR) purification kit (Qiagen). Labeled test and reference DNAs were mixed and dissolved in hybridization solution (Macrogen) containing 100 μl Cot-1 DNA solution and 4 μl yeast tRNA solution (Macrogen). The array CGH was provided by Macrogen MAC Array KARYO 4000. This array slide had 4030 BAC clone DNAs in duplicate and covered the entire human genome with 1-Mbp resolution. The hybridization-to-wash procedure was carried out by using a Hybristation (Digilab Inc., Holliston, MA). Hybridization was carried out at 37°C for 48–72 h on the Hybristation with continuous agitation. The wash procedure was as follows: 50% formamide/2× standard saline citrate (SSC) at 46°C for 15 min, followed by 0.1% SDS/2× SSC at 46°C for 30 min, PN buffer (0.1 M Na_2PO_4/0.1% NonDiet P-40, Nakarai Tesque, Kyoto, Japan) at 37°C for 15 min, and 2× SSC at 37°C for 5 min. The array slides were scanned at 532 and 635 nm by using a GenePix4000A (Molecular Devices, Sunnyvale, CA) and analyzed by Mac Viewer software (Macrogen). The Mac Viewer software analyzed the results as follows: (1) averaged the fluorescence ratios of the replicates and calculated

the standard deviation (SD), (2) rejected individual spot data based on several criteria including weak fluorescent signals, (3) adjusted Cy5/Cy3 ratios such that ratios of the normal genomic regions were always equal to 0, despite variations in dye labeling efficiency, and (4) plotted data relative to the position of the clones on the human genome, according to July 2003, University of California, Santa Cruz cartography. In this study, all BAC aCGH analyses were confirmed to calibrate by the hybridization of the normal male DNA versus normal female DNA. The entire SD value of the $\log_2$ ratio calculated for chromosomes 1–22 was 0.07. Accordingly, DNA copy number abnormalities were defined as more than three times higher than the SD in order to account for experimental errors. For this research, a $\log_2$ ratio of 0.3 was employed to indicate abnormal differences, with the normalized $\log_2$ ratio of fluorescence intensity of over 0.3 being taken as gain and one of below −0.3 as loss.

Results and discussions

Evaluation of HeLa cells

To evaluate the chromosomal stability and instability of HeLa cells, we analyzed three different HeLa cells by BAC aCGH, as shown in Fig. 1. In the case of HeLa-A, DNA was directly extracted from ATCC HeLa cells without cultivation. HeLa-B and HeLa-C were cultured for different periods of time prior to DNA extraction (HeLa-B for 122 passages and HeLa-C for 150 passages). Novel DNA copy number loss occurred at chromosomes 3 and 13 in HeLa-C [Fig. 1a, b(i), (iii), respectively]. Moreover, at 9p13.1–p24.3, on the short arm of chromosome 9, CGH profiles showed a tendency of the DNA copy number to decrease with increased passage time [Fig. 1a, b(ii)]. Similar results were obtained for the entire regions of chromosome 1 [Fig. 1a(*)]. In contrast, the CGH profiles showed a tendency for the DNA copy number for the entire regions of both chromosomes 21 and 22 to increase with increased passage time [Fig. 1a(*)]. Additionally, Table 1 summarizes the average of $\log_2$ ratios for the above-mentioned regions obtained from BAC aCGH analysis. These results indicate that chromosomal instability including DNA copy number alterations was generated by long-term culture of HeLa cells. HeLa-C, in comparison to HeLa-A, would be distinguished as a variant of HeLa cells or might be a different cell. To summarize our analysis using BAC aCGH, continuous cultivation of HeLa cells caused a significant change at the chromosomal level. Until now, chromosomal changes in cultured cells have been recognized only empirically. If a chromosomal change occurs, it will result in a significant change at the expression level. For scientific research using cultured cells, such a change is extremely critical. Based on our present findings, we stress the importance of validation of experimental cultured cells even at the chromosomal level.

Evaluation of Caco-2 cells

This colon cancer cell line is well known to be a heterogeneous cell line and to differentiate spontaneously into small intestinal epithelial cells after its cultures have reached confluence [18–20]. Such differentiated Caco-2 cells can be cultured as monolayers on permeable filters and correlate well with the absorption system of normal intestinal cells. Therefore, Caco-2 cells are used industrially as a simulation model of intestinal drug absorption in drug discovery [15]. As described above, HeLa cells displayed chromosomal instabilities including DNA copy number alterations in a passage time-dependent manner. To evaluate the chromosomal stability including DNA copy number aberrations of Caco-2 cells, we analyzed Caco-2 cells under several different conditions by using BAC aCGH. The CGH profile for Caco-2-a, which was used as the control, is shown in Fig. 2. These cells showed no significant difference in comparison to Caco-2 cells purchased from the European Collection of Cell Cultures (ECACC, Wiltshire, UK) or from DS Pharma Biomedical Co., Ltd. (Osaka, Japan; data not shown). Caco-2-b cells, which were analyzed at passage number 63, and Caco-2-c cells, which had been cultured on the microporous membranes, showed no remarkable differences in CGH profile in comparison to Caco-2-a. Other culture conditions, such as fewer passage times than the 63 passages for Caco-2-b and use of larger diameter membrane than that used for Caco-2-c, gave similar CGH profiles (data not shown). These results indicate that the Caco-2 cell line, in comparison to the HeLa cell, is a chromosomally stable cell line, even though it was established from a cancer cell. Therefore, the Caco-2 cell line would be considered a suitable cell line for use in a validation system of intestinal drug absorption, as verified from the aspect of chromosomal stability assessed by BAC aCGH.

The ACBRI-519 cell line, which was established from normal human intestinal epithelial cells, was regarded as an alternative of the Caco-2 cell line. CGH profiles showed no significant differences between ACBRI-519 and Caco-2 cells, as also shown in Fig. 2. According to the result of BAC aCGH, ACBRI-519 and Caco-2 cells would be regarded as the same cell line. Indeed, Yamamoto et al. [21] reported that the IL-8 response to oxidative stress was almost the same between Caco-2 cells and ACBRI-519 cells. Thus, BAC aCGH can be used to recognize and to distinguish cell lines.

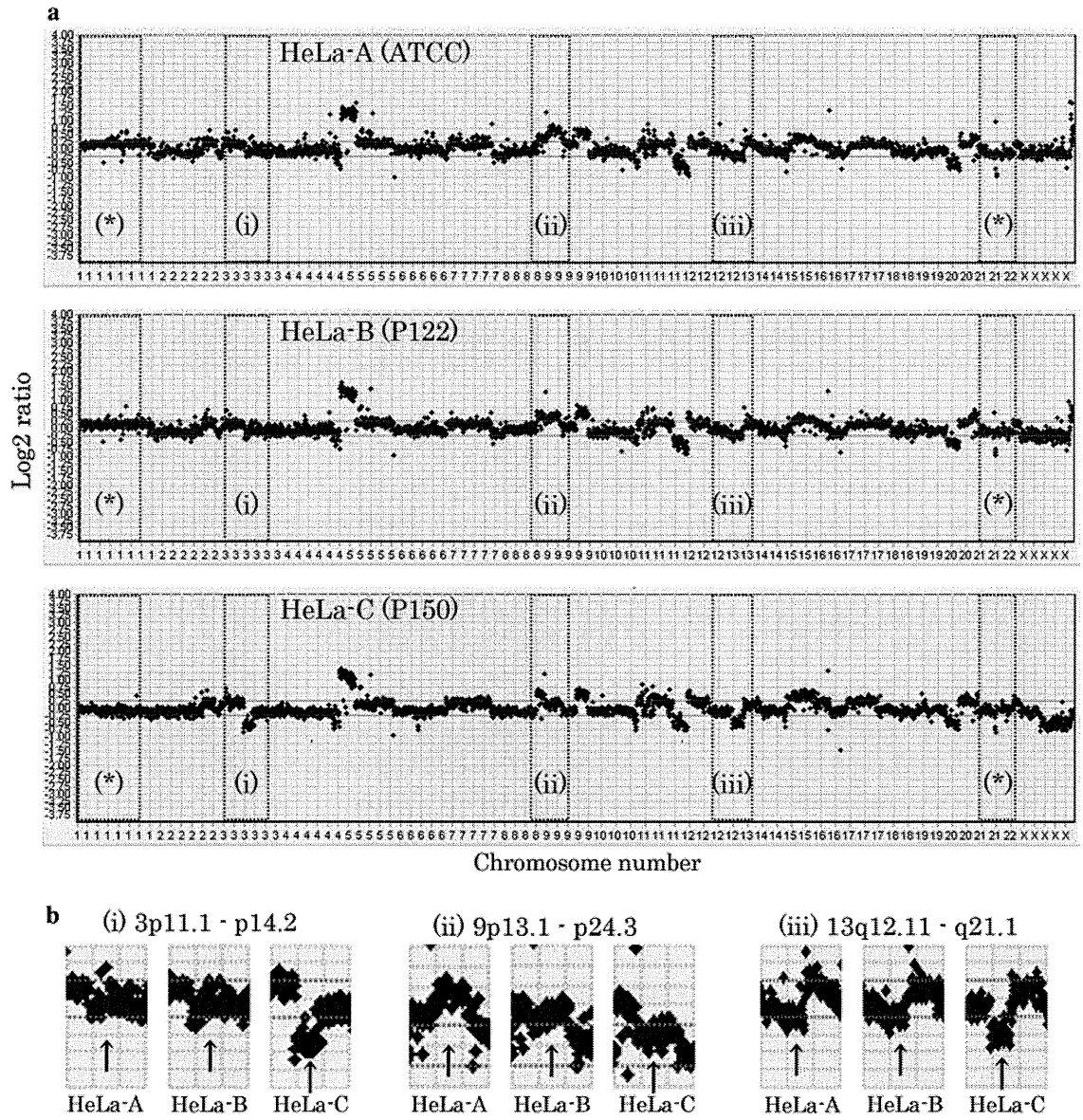

Fig. 1 BAC aCGH profiles of three HeLa cells. **a** *Upper panel* BAC aCGH profile of HeLa-A (ATCC), *middle panel* HeLa-B (JCRB, after 122 passages), *lower panel* HeLa-C (JCRB, after 150 passages). (*i*)–(*iii*) Correspond to **b**(*i*) to (*iii*), respectively. An *asterisk* indicates a tendency for DNA copy number alterations. The *ordinate* indicates the $\log_2$ ratio of Cy5/Cy3 and abscissa, the chromosome number (also applies to Figs. 1b, 2, 3). **b** Three remarkable regions of DNA copy number aberrations. *Arrows* point to regions of remarkable DNA copy number loss

Evaluation of MSCs

MSCs have been widely used clinically in the field of regenerative medicines; for instance, they are used for the treatment of osteoarthritis, bone tumor, acute myocardial infarction, and graft-versus-host disease [16, 17, 22, 23]. Because the tumorigenesis of MSCs is still a controversial issue, the safety evaluation of MSCs is very important [9–11]. BAC aCGH is a powerful method for detecting DNA copy number aberrations, which are strongly associated with tumorigenesis. In this study, we analyzed MSCs that already had been used clinically without tumor formation for osteoarthritis patients [16]. As shown in Fig. 3, the CGH profiles of MSC-1, MSC-2, and MSC-3 followed the baseline linearly; the SD values for these CGH profiles were 0.028 ± 0.060 for MSC-1, 0.043 ± 0.072 for MSC-2, and 0.029 ± 0.063 for MSC-3. In the case of MSC-4, which was passaged three more times than MSC-3, it also followed the baseline linearly (SD value was 0.018 ± 0.073). These results indicate that these MSCs did not have any chromosomal instability including DNA copy number aberrations. Therefore, BAC aCGH was able to confirm the

Table 1 Average of $\log_2$ ratios for the regions showing DNA copy number alterations in three types of HeLa cells

Region	Average of $\log_2$ ratio					
	Chr. 1	3p11.1–p14.2	9p13.1–p24.3	13q12.11–q21.1	Chr. 21	Chr. 22
HeLa-A (ATCC)	0.120	−0.114	0.581	−0.248	−0.180	−0.145
HeLa-B (P122)	0.080	−0.154	**0.337**	−0.245	−0.170	−0.105
HeLa-C (P150)	**−0.126**	**−0.635**	**0.126**	**−0.511**	*−0.146*	*−0.029*
Number of clones	299	34	63	50	68	100

Chr. indicates whole region of the chromosome; P122 and P150, analysis after 122 and 150 passages, respectively; number of clones, the number of BAC clones in the corresponding region; bold type, the value of the $\log_2$ ratio decreased in comparison to that for HeLa-A; italic type, the value of the $\log_2$ ratio increased in comparison to that for HeLa-A

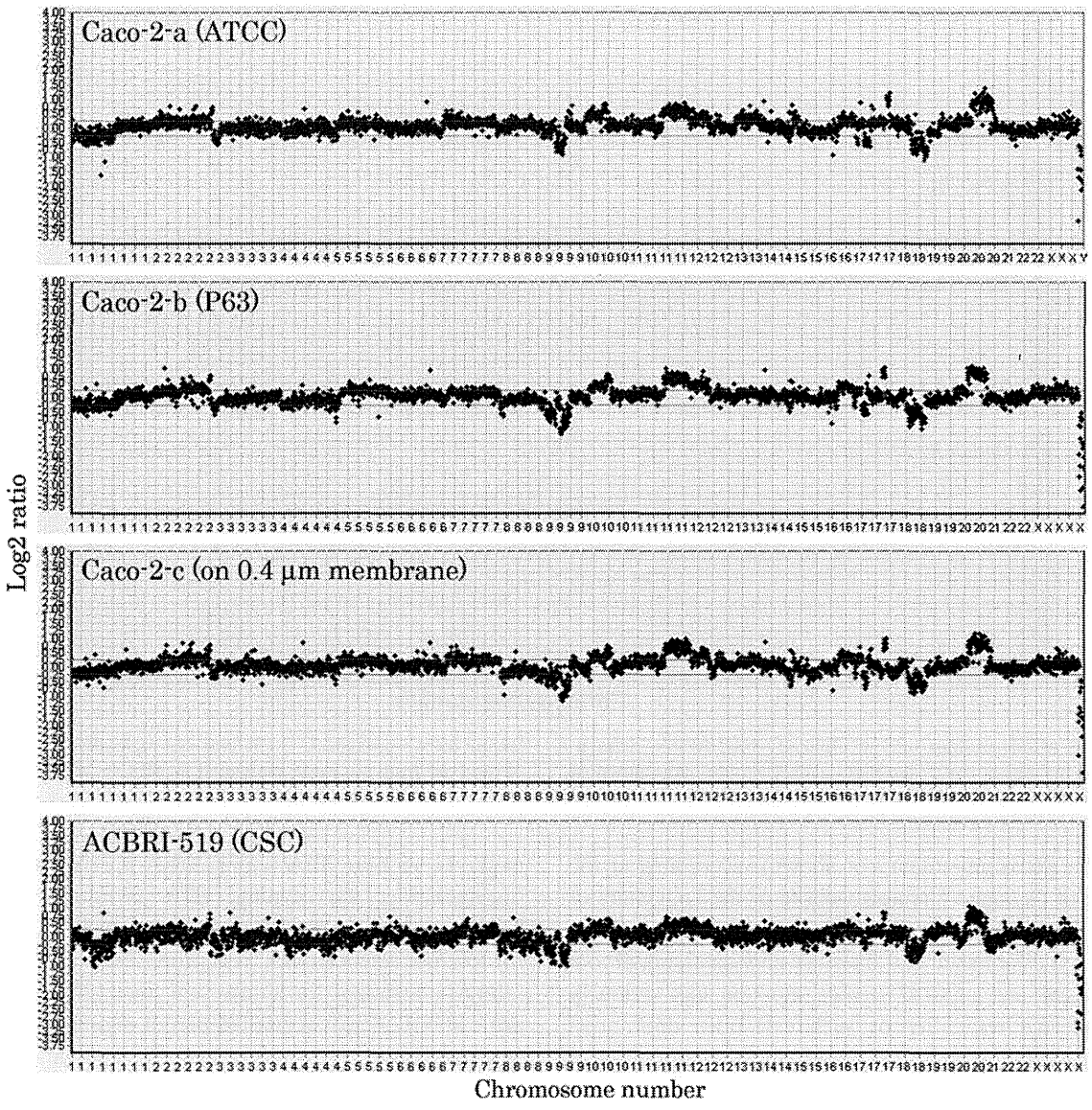

Fig. 2 BAC aCGH profiles of 3 Caco-2 cells and ACBRI-519 cells. Sequentially from the upper panel are the BAC aCGH profile of Caco-2-a (ATCC), -b (after 63 passages), -c (cultured on 0.4-μm diameter microporous membrane), and ACBRI-519 (Cell System Corp.)

safety of these MSCs at the genome level, especially DNA copy number change, which correlates well with tumorigenesis. In Fig. 3, several BAC clones that were outside the normal range (considered as normal from −0.3 to 0.3, see "Materials and methods") were possible copy number variants (CNV). These clones were confirmed as CNV loci

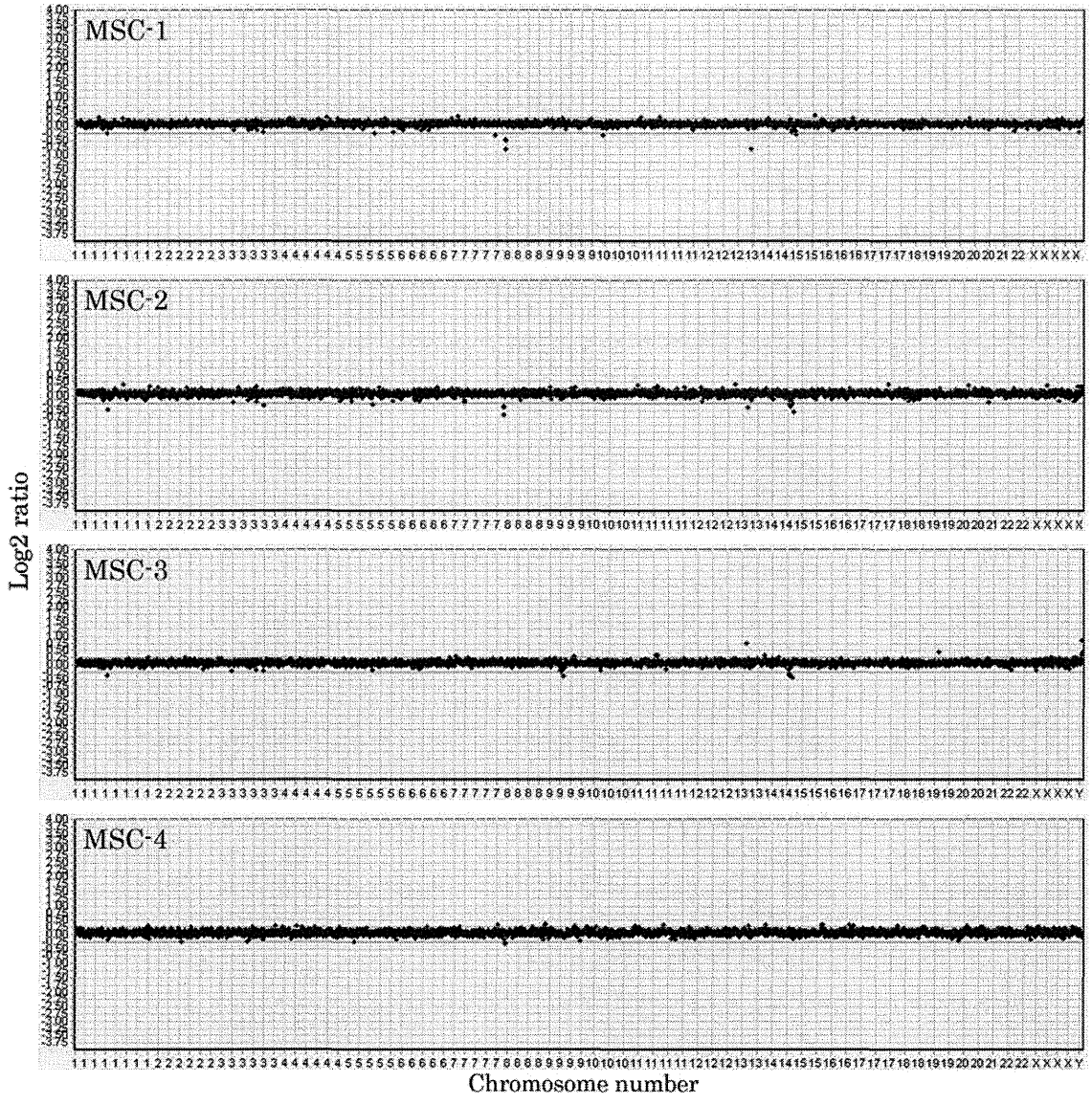

Fig. 3 BAC aCGH profiles of four clinically used MSCs. Sequentially from the upper panel the BAC aCGH profiles are shown of MSC-1 (69-year-old female after three passages), -2 (16-year-old female after 3 passages), -3 (34-year-old male after 4 passages), and -4 (derived from the same individual as MSC-3, after 7 passages)

or in their proximity (data not shown), according to the Database of Genomic Variants website of the University of Toronto (http://projects.tcag.ca/variation/).

Although established cell lines are now an essential tool in biological and clinical studies, no one has seriously questioned the reliability of such cell lines until now. Actually, most investigators have noted morphological and/or biological alterations of cell lines during long-term culture. For coping with such alterations, these cell lines have been discarded and renewed from frozen stocks after a certain number of passages. However, the substantial alterations caused by long-term culture have not been seriously considered. Validation to detect cross contamination of cell lines has been made by using various methods, such as HLA typing, DNA polymorphism, DNA fingerprinting, karyotyping, STR profiling, and SNPs [24–29]. In particular, STR profiling, which was developed for forensic sciences [30], was proposed as an international reference standard for human cell lines [28]. These methods can only detect at limited partial regions of the human genome. However, tumorigenesis is known to correlate with various chromosomal instabilities including DNA copy number changes throughout the entire human genome. Therefore, to avoid overlooking the possibility of tumorigenesis, it is necessary to validate cells by using BAC aCGH, as it can analyze all regions in the entire human genome. In this study, we investigated chromosomal stability and instability of established cell lines,

HeLa cell, Caco-2 cells, and MSCs derived from normal human bone marrow by performing BAC aCGH. Our results indicate that BAC aCGH is a suitable tool for validation of cell lines. Thus, we propose that BAC aCGH is a superior method for evaluation of the genomic stability of established cell lines as well as various kinds of cells and suggest that all kinds of cellular investigations should include validation of chromosomal stability by performing BAC aCGH.

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Growth factor-defined culture medium for human mesenchymal stem cells

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ABSTRACT Human bone marrow-derived mesenchymal stem cells (hMSCs) are potential cellular sources of therapeutic stem cells as they have the ability to proliferate and differentiate into a wide array of mesenchymal cell types such as osteoblasts, chondroblasts and adipocytes. hMSCs have been used clinically to treat patients with graft *vs.* host disease, osteogenesis imperfect, or alveolar cleft, suggesting that transplantation of hMSCs is comparatively safe as a stem cell-based therapy. However, conventional culture medium for hMSCs contains fetal bovine serum (FBS). In the present study, we developed a growth factor-defined, serum-free medium for culturing hMSCs. Under these conditions, TGF-β1 promoted proliferation of hMSCs. The expanded hMSC population expressed the human pluripotency markers SSEA-3, -4, *NANOG, OCT3/4* and *SOX2*. Furthermore, double positive cells for SSEA-3 and a mesenchymal cell marker, CD105, were detected in the population. The potential to differentiate into osteoblasts and adipocytes was confirmed. This work provides a useful tool to understand the basic biological properties of hMSCs in culture.

KEY WORDS: *mesenchymal stem cell, serum-free culture, TGF-β1*

Introduction

Bone marrow-derived cells can differentiate into osteoblasts *in vitro* and *in vivo* (Friedenstein *et al.*, 1966) and thus are considered a useful source of stem cells for bone regeneration. Recently, many studies have reported that human bone marrow contains a distinct cell fraction referred to as multipotent mesenchymal stem cells (hMSCs) which can give rise to a wide array of mesenchymal cell types, including bone, fat, and cartilage"(Pittenger *et al.*, 1999). However, hMSCs can differentiate along some ectodermal and endodermal cell lineages such as neuronal cells and liver cells (Pittenger *et al.*, 1999; Dezawa *et al.*, 2004; Dezawa *et al.*, 2005). Further, a recent study reported that hMSCs have the ability to generate the multiple cell types derived from the three embryonic germ layers (Kuroda *et al.*, 2010). It has been estimated that hMSCs comprise about 0.001 to 0.01% of total bone marrow mononuclear cells (Pittenger *et al.*, 1999). For use in cell-based therapies, hMSC populations require extensive *in vitro* expansion to obtain sufficient numbers. The conventional culture medium for hMSCs is composed of a basal nutrient medium supplemented with fetal bovine serum (FBS) (Haynesworth *et al.*, 1992; Lennon DP, 1996). Although these traditional culture conditions provide robust undifferentiated hMSC expansion, the ill-defined components of FBS is undesirable for clinical applications and also hampers analysis of the cell biological mechanisms that control cell behavior.

Abbreviations used in this paper: hES cells, human embryonic stem cells; hMSCs, human mesenchymal stem cells.

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We and others previously described serum-free media consisting of minimum essential components suitable to propagate and accurately analyze the characteristics of differentiated cells (Hayashi and Sato, 1976; Furue and Saito, 1998; Sato *et al.*, 2002; Furue *et al.*, 2005; Furue *et al.*, 2008; Hayashi *et al.*, 2010). One of these media, hESF9, supports the serial cultivation of undifferentiated human embryonic stem (hES) cells in the absence of feeder cells and thus provides an experimental system for elucidating cellular responses to specific environmental stimuli (Furue *et al.*, 2008; Na *et al.*, 2010). For example, either FGF-2 or heparin promotes proliferation of hES cells in a concentration-dependent manner although these effects were not detected under conventional culture conditions. Thus, a defined serum-free medium consisting of minimum essential components should be useful in elucidating hES/iPS cell responses to specific cues that control self-renewal, differentiation, and lineage selection (Furue *et al.*, 2010).

Because hMSCs have multipotent properties similar to hES cells, we speculated that hMSCs should be able to grow in similar culture conditions as hES cells. In the present study, we demonstrated that addition of TGF-β1 to the defined serum-free medium for hES cells supports the robust proliferation of hMSCs. The hMSC population expanded in the absence of serum expressed the mesenchymal cell markers CD44, CD73, CD90, and CD105. Further, they expressed human pluripotency surface markers, SSEA-3, -4, TRA-2-54, and also the transcription factors of *NANOG*, *OCT3/4*, and *SOX2*. We show that the serum-free expanded hMSCs can differentiate into osteoblasts and adipocytes. This work sets the stage for serum-free hMSC cell culture and thereby provides a useful tool to understand the basic biological characteristics of hMSCs.

Results

In this study we used a human bone marrow-derived hMSC line designated UE7T-13 (JCRB 1154). The life span of these cells was prolonged by infecting them with a retrovirus containing human papillomavirus E7 and telomerase reverse transcriptase (hTERT) cDNAs (Mori *et al.*, 2005; Shimomura *et al.*, 2007; Ishii *et al.*, 2008; Takeuchi *et al.*, 2007). We first tested the ability of hESF9 medium, which we had developed for use with hES cells, to support the growth of UE7T-13 cells. The cells were harvested using trypsin/EDTA, from cultures in conventional medium containing 10% FBS (POWERDBY10) and transferred to 0.1% gelatin-coated dishes in hESF9 medium. However, UE7T-13 cell growth was quite slow. We then investigated the effects of various growth factors on proliferation of the cells. UE7T-13 cells were seeded on 0.1% gelatin in hESF9 in the absence of FGF-2 and heparin (hESF9(-/-)), containing increasing concentrations of FGF-1, FGF-2, TGF-β1, activin A, or leukemia inhibitory factor (LIF) (Fig. 1). Both FGF-1 and FGF-2 promoted UE7T-13 proliferation in a dose-dependent manner, and the greatest effect was seen at 10 ng/ml FGF-2. Neither LIF nor activin A affected on UE7T-13 cell proliferation, but TGF-β1 slightly stimulated UE7T-13 proliferation. Next all five factors (FGF-1, FGF-2, TGF-β1, activin A, and LIF) or four factors with increasing concentrations of heparin were added to UE7T-13 cultures (Fig. 2). When either FGF-2 or TGF-β1 was withdrawn from the cultures, the cell numbers decreased significantly. Heparin promoted cell proliferation in a dose-dependent manner. This result suggested that addition of FGF-2 and TGF-β1 to hESF9(-/-) medium, is critical for UE7T-13 proliferation, and heparin also enhanced cell growth. hESF9 medium supplemented with TGF-β1 was designated hESF10.

L-ascorbic acid-2-phosphate (Asc 2-P) in hESF9 medium supported hES cells. However, it is known to promote hMSC cell differentiation into osteoblasts. Therefore, we examined whether the presence of Asc 2-P in hESF10 medium promoted osteoblastic differentiation of UE7T-13 cells. We analyzed the expression of *bone sialoprotein* (*IBSP*), *osteocalcin* (*BGP*), *osteonectin* (*SPOCK2*), and *osteopontin* (*SPP1*) in UE7T-13 cell cultured in hESF10 with or without Asc 2-P and in conventional medium (Fig. 2). These osteoblast genes were expressed at significantly lower levels in cells cultured in the serum-free media than in those cultured in the conventional medium. These results suggest that the serum-free medium is suitable for hMSC maintenance. *IBSP* gene expression was higher in the cells cultured in the

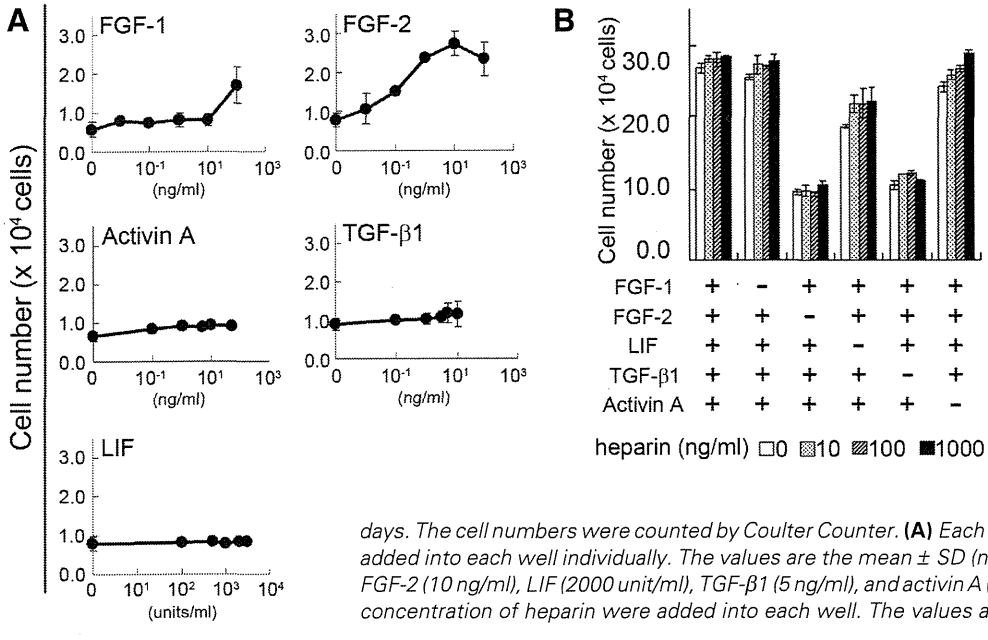

Fig. 1. Effect of growth factors on UE7T-13 cell proliferation in defined serum-free culture conditions. *After the UE7T-13 cell grown in the conventional culture conditions (POWERDBY10) were cultured in hESF9(-/-) overnight, the cells were seeded in a 24-well plate coated 0.1% gelatin in hESF9(-/-) at 1 x10⁴ cells per well and cultured for 6 days. The cell numbers were counted by Coulter Counter.* **(A)** *Each growth factor at indicated concentration was added into each well individually. The values are the mean ± SD (n=3).* **(B)** *All five factors of FGF-1 (100 ng/ml), FGF-2 (10 ng/ml), LIF (2000 unit/ml), TGF-β1 (5 ng/ml), and activin A (10 ng/ml) or four factors of them with varying concentration of heparin were added into each well. The values are the mean ± SD (n=3).*

presence of Asc 2-P. These results suggested that Asc 2-P promoted differentiation of UE7T-13 cells into osteoblasts. We removed Asc 2-P from hESF10 medium for hMSCs, and designated the new formulation D-hESF10.

To confirm the characteristics of UE7T-13 cells expanded in the absence of serum, we performed flow cytometry with antibodies to markers for hMSCs and pluripotent cells (Fig. 3A). Cells grown in D-hESF10 medium were positive for CD44, CD73, CD90, CD105, and TRA-2-54 (tissue non-specific alkaline phosphatase antibody), but negative for CD45 (a marker of all hematopoietic cells) and CD56 (a neural cell adhesion molecule). We further stained the cells with antibodies to CD105 and SSEA-3 (Fig. 3B). The immunocytochemical analysis showed that SSEA-3$^+$/CD105$^+$ double positive cells were present in the UE7T-13 population grown in D-hESF10 although cells positive for either CD105 or SSEA-3 were also detected in the population. The cell growth rate in D-hESF10 was comparable to that in conventional culture conditions (Fig. 4).

We subsequently examined the properties of UE7T-13 cells serially passaged in D-hESF10 medium. The morphology of serum-free expanded UE7T-13 cell populations was comparably small, spindle-shaped cells compared with that in conventional medium (Fig. 5A). The expression of hMSC and hES cell pluripotency markers were determined by real-time PCR analysis (Fig. 5B) in UE7T-13 cells cultured for 4 passages in D-hESF10 medium. The expression of hMSC markers, *CD105*, *THY1*, and *integrinβ1* (*ITGB1*), and the hES cell pluripotency markers, *OCT3/4* (*POU5F1*) and *NANOG* were similar in the cells cultured in D-hESF10 compared with those in the cells cultured in conventional culture conditions. *SOX2* expression was significantly higher in cells cultured in D-hESF10 compared with cells cultured in conventional culture conditions. On the other hand, the expression levels of *IBSP*, *BGP*, *SPOCK2*, and *SPP1* were significantly lower in cells cultured in D-hESF10 compared with those in the cells cultured in conventional culture conditions. These results suggest that serum-free expanded UE7T-13 cells retain an undifferentiated phenotype.

We determined the differentiation capacity of the serum-free expanded UE7T-13 cells. After the UE7T-13 cells were cultured in D-hESF10 for 7 passages, the cells were cultured in medium designed to induce differentiation into osteoblasts or adipocytes (Fig. 6). Culturing in osteoblastic differentiation medium induced the formation of nodules that stained positive with Alizarin red, suggesting that the cells had the potential to differentiate into osteoblasts. When the cells were cultured in

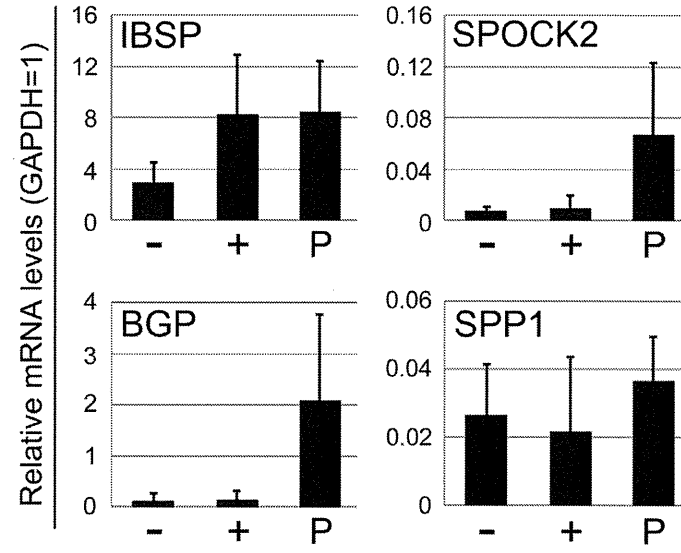

Fig. 2 (above). The effect of culture conditions on osteoblastic marker expression. *The gene expression in the cells cultured on gelatin in hESF10 without (-) or with (+) Asc 2-P for 6 days, in comparison with the cells grown in POWERDBY10 (P) was analyzed by the quantitative RT-PCR. The gene expression was normalized by the amount of* GAPDH. *The values are the mean ± SD (n=3).*

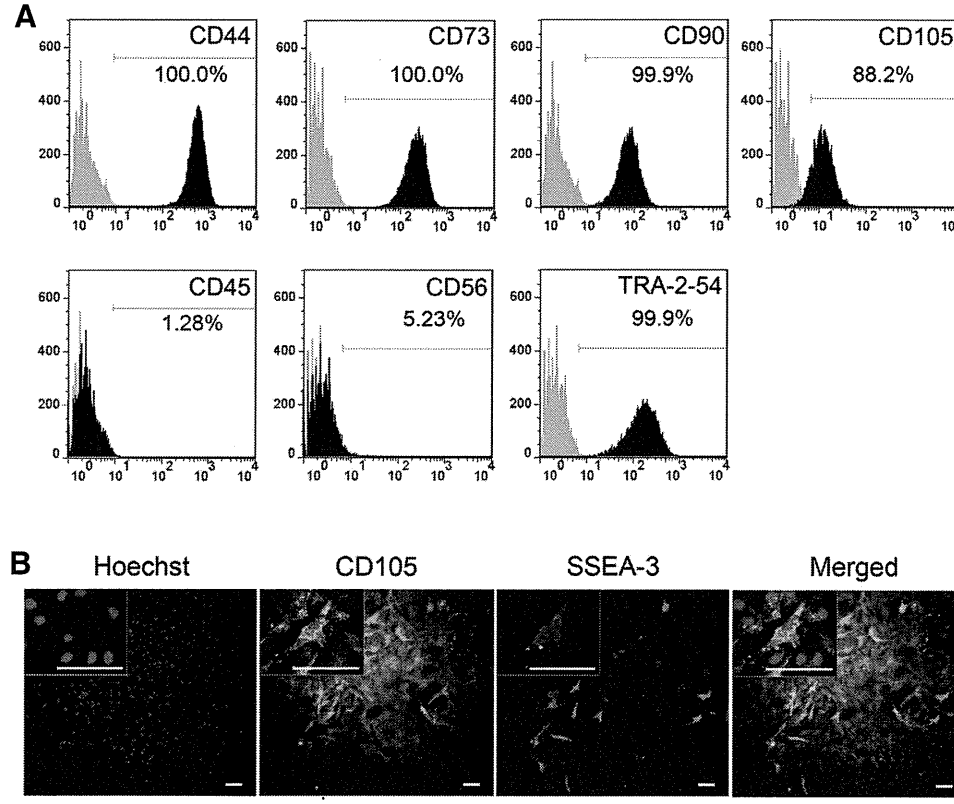

Fig. 3. Expression of hMSC markers in UE7T-13 cells. (A) *Flow cytometric profiles for CDs in UE7T-13 cells. hMSC marker expression in UE7T-13 cells cultured on gelatin in D-hESF10 for 4 days was analyzed by flow cytometric analysis. Antigen histogram (black); control histogram (gray); the horizontal bar indicates the gating used to score the percentage of antigen-positive cells.* **(B)** *Immunocytochemical analysis of SSEA-3 and CD105 expression in UE7T-13 cells cultured on gelatin in D-hESF10 for 4 days. Scale bars, 100 μm.*

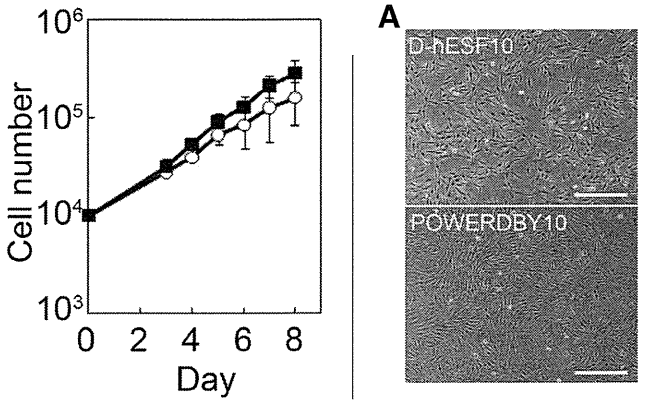

Fig. 4 (above left). A comparison of the growth of different UE7T-13 cells in the defined serum-free medium and conventional culture conditions. *The cells were seeded in a 24-well plate coated with gelatin in D-hESF10 (open circle), or in a 24-well plate in POWERDBY10 (closed square) at a cell density of 1x10⁴ cells per well. Cell numbers were counted every day. The values are the mean ± SD (n=3).*

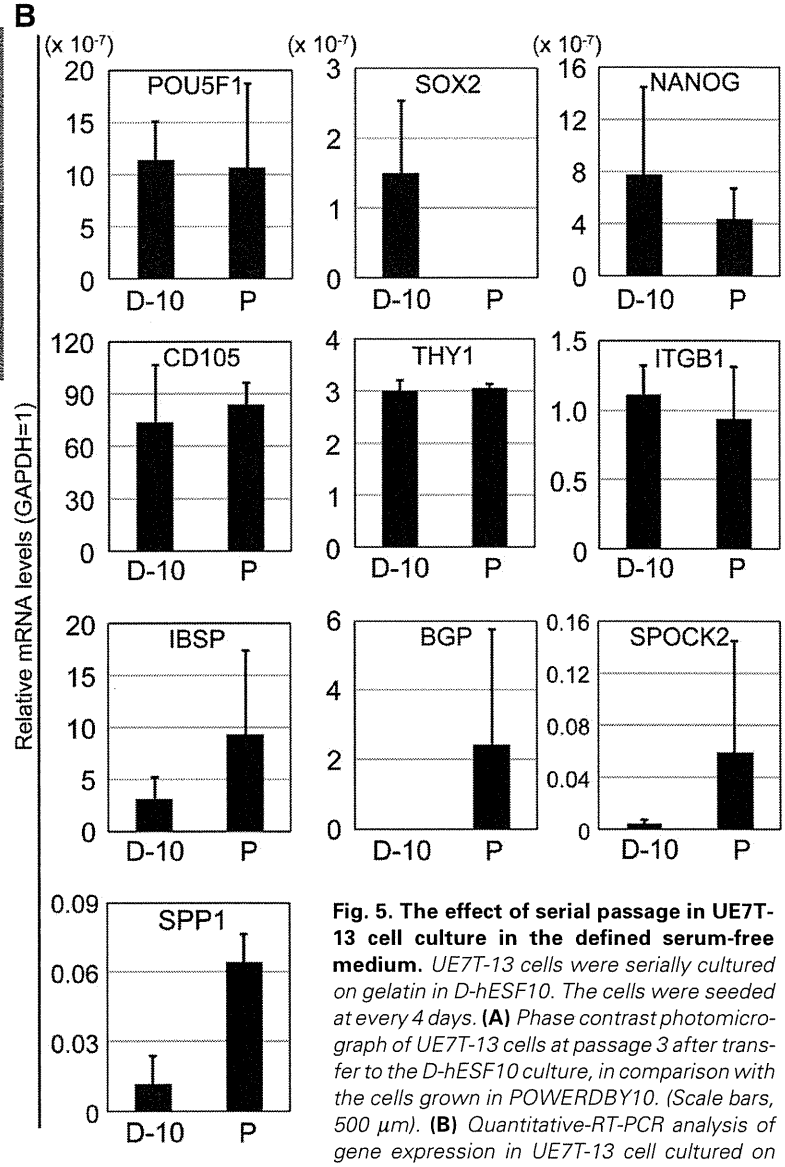

Fig. 5. The effect of serial passage in UE7T-13 cell culture in the defined serum-free medium. *UE7T-13 cells were serially cultured on gelatin in D-hESF10. The cells were seeded at every 4 days.* **(A)** *Phase contrast photomicrograph of UE7T-13 cells at passage 3 after transfer to the D-hESF10 culture, in comparison with the cells grown in POWERDBY10. (Scale bars, 500 μm).* **(B)** *Quantitative-RT-PCR analysis of gene expression in UE7T-13 cell cultured on gelatin in D-hESF10 at passage 4 (D-10), in comparison with the cells grown in POWERDBY10 (P). The name of each gene is noted in each bar graph. Gene expression was normalized with respect to* GAPDH. *The values are the mean ± SD (n=3).*

adipocytic differentiation medium, Oil red O-positive cells appeared. Taken together these results suggest that the serum-free expanded UE7T-13 cells have maintained the capacity to differentiate into osteoblasts or adipocytes.

Discussion

Developing clinical serum-free media for maintaining and expanding human stem cells is a major research topic in regenerative medicine. Our current results indicate that it is possible to culture hMSCs on gelatin in a defined medium, designated D-hESF10, in which human recombinant insulin, human transferrin, a low concentration of fatty acid-free bovine albumin conjugated with oleic acid, FGF-2, and TGF-β1 are the protein components. The basal medium ESF was developed for mouse ES cells (Furue *et al.*, 2005). For hES cell culturing, N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid (HEPES) was removed from ESF but Asc 2-P was added (Furue *et al.*, 2008). For propagating hMSCs, Asc 2-P was removed from the hES cell culture medium because we found that Asc 2-P increased osteoblastic marker expression in hMSCs. These findings indicated that signaling by Asc 2-P in hMSCs is different from that in hES cells.

FGF-2 is a heparin-binding growth factor which stimulates the proliferation of a wide variety of cells. The biological activity of FGF-2 is efficient in the concentration range of 0.1 to 10.0 ng/ml. Addition of FGF-2 has been shown to increase the growth rate and life span of hMSCs from different species (Tsutsumi *et al.*, 2001; Benavente *et al.*, 2003), suggesting that FGF-2 play an important role in self-renewal of hMSCs. In hES cells, FGF-2 is a crucial to maintain the undifferentiated state (Amit *et al.*, 2004; Hoffman and Carpenter, 2005). We previously reported that FGF-2 at 10 ng/ml together with heparin supported the cell proliferation of hES cells in serum-free without feeders (Furue *et al.*, 2008). In this study, we found that FGF-2 at 10 ng/ml together with heparin supported the cell proliferation of hMSCs in a serum-free medium. These findings suggest that they share the same signal pathway to support self-renewal. Heparin at 1 mg/ml promoted hMSC cell proliferation, and we previously reported that heparin at 1 mg/ml inhibited hES cell proliferation. Thus the sensitivity to heparin is different between hMSCs and hES cells.

The TGF-β1 pathway has been reported to be important in hMSC differentiation into the osteogenic and chondrogenic lineages (Li and Xu, 2005; Kulterer *et al.*, 2007). While we have shown that TGF-β1 alone did not promote cell proliferation of hMSCs, the combination with FGF-2 and heparin enhanced cell proliferation of hMSCs. Chase *et al.* reported the combination of TGF-β1, FGF-2, and PDGF-BB in a commercial serum-free medium for the expansion of hMSCs although the optimal concentrations of these factors were not disclosed. The cell growth rate in D-hESF10 medium was similar with that in the conven-

tional culture conditions suggesting that addition of TGF-β1 and FGF-2 is sufficient to replace serum in supporting hMSC cell growth. A culture medium consisting of the minimum components necessary to support survival and proliferation would be beneficial to understand the characteristics of naïve hMSCs. Therefore, we think that addition of PDGF-BB is not crucial for an hMSC culture medium.

Several studies reported that two distinct cell morphologies are seen in early-passage hMSC cultures: small, spindle-shaped cells that are rapidly self-renewing and large, flat cells that replicate slowly and appear more mature (Mets and Verdonk, 1981; Colter *et al.*, 2001; Sekiya *et al.*, 2002). The morphology of serum-free expanded UE7T-13 cell population contained comparably small, spindle-shaped cells. However, specific undifferentiated markers of hMSCs have not been identified yet (Pochampally *et al.*, 2004). Further, although the cells are cloned, cells within an individual colony are heterogeneous in morphology, growth rates, and efficiency with which they differentiate (Mets and Verdonk, 1981; Bruder *et al.*, 1997; Colter *et al.*, 2001). The International Society for Cellular Therapy (ISCT) has proposed three criteria to define hMSCs (Dominici *et al.*, 2006). hMSC population must be positive at least for several antigens such as CD105, CD73, and CD90, and negative for CD45. CD105 is usually used to identify an hMSC population. Many studies reported that hMSCs also expressed hES cell pluripotency markers, SSEA-3, -4, NANOG, OCT3/4, and alkaline phosphatase (Pochampally *et al.*, 2004; Roubelakis *et al.*, 2007; Battula *et al.*, 2008; Conrad *et al.*, 2008; Pang *et al.*, 2010). We also detected the expression of NANOG, OCT3/4, and SOX2. These findings suggested that hES cell pluripotency markers may be universal stem cell markers in humans. Dezawa's group recently reported that double positive CD105 and SSEA-3 cells have the ability to generate multiple cell types derived from the three embryonic germ layers (Kuroda *et al.*, 2010). We also confirmed the existence of CD105 and SSEA-3 double positive cells in the hMSC population expanded in D-hESF10. In this study, we confirmed the differentiation potential of hMSCs to generate osteoblasts or adipocytes, but in the future we will examine the ability of hMSCs to generate cells from all three germ layers.

To facilitate the transition of human stem cell biology from basic research to clinical application all the components of maintenance and differentiation media should be publicly disclosed so they can be evaluated by many researchers. A commercial xeno-free serum-free medium for hMSCs was reported recently (Chase *et al.*, 2010). However, the non-disclosure of components is problematic as the medium formulation cannot be usefully modified or improved. Because all the components of D-hESF10 medium are disclosed here, the medium can be modified to study signaling pathways involved in maintaining multipotency and to develop differentiation protocols.

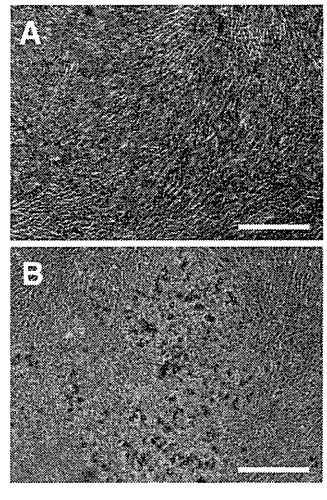

Fig. 6. The differentiation ability of UE7T-13 cell grown in the defined medium. *The UE7T-13 cells were serially cultured in D-hESF10 at passage 7, and then cultured in the differentiation medium.* **(A)** *Osteoblastic differentiation was induced in osteoblastic medium for 20 days. The nodules were stained with Alizarin Red S (red).* **(B)** *Adipocytic differentiation was induced in adipocytic medium for 24 days. The cells were stained by Oil red O staining (red). Scale bars: 500 μm.*

Materials and Methods

Cell Cultures

An immortalized hMSC line UE7T-13 (Mori *et al.*, 2005) (JCRB 1154, JCRB Cell Bank, Osaka, Japan) was used in this study. Cells were maintained on 100 mm dish (BD Falcon, Oxnard, CA) in POWERDBY10 (MED-SHIROTORI, Tokyo, Japan) that was also used in the experiments as a control medium. The cells were harvested with 0.25% trypsin in 1 mM EDTA-4Na.

Serum-free Cell Culture Media

hESF9 comprises ESF basal medium (Furue *et al.*, 2005) without HEPES supplemented with nine defined factors: Asc 2-P, 6-factors (human recombinant insulin, human transferrin, 2-mercaptoethanol, 2-ethanolamine, sodium selenite, oleic acid conjugated with fatty acid-free bovine serum albumin (FAF-BSA)), bovine heparan sulfate sodium salt, and human recombinant FGF-2 (Sigma, St. Louis, MO), as described previously (Furue *et al.*, 2008) (Supplementary Table 1). ESF basal medium without HEPES supplemented with Asc 2-P (hESF-GRO), and ESF basal medium without HEPES and Asc 2-P (hESF-DIF) were purchased by the Cell Science & Technology Institute (CSTI, Sendai, Japan). All other reagents were from Invitrogen (Carlsbad, CA) and Sigma. D-hESF10 medium consists of hESF-DIF medium supplemented with 6-factors, FGF-2, heparin, and TGF-β1 (R&D Systems, Minneapolis, MN). To harvest cells, 0.25% trypsin in 1 mM EDTA-4Na was used and the trypsin was inactivated with 0.1% soybean trypsin inhibitor (Sigma). For differentiation into osteoblasts or adipocytes, the cells were cultured according to the instruction by the suppliers (Lonza, Basel, Switzerland). The differentiated cells were stained by Alizarin Red S (Wako Pure Chemical Industries, Osaka, Japan) or Oil Red O (Wako).

Cell proliferation

Before the serum-free experiments, cells grown in POWERDBY10 were incubated by in hESF9 medium without heparin and FGF-2 (hESF9(-/-)) overnight to starve the effect of serum. Cells were replaced at the cell density of $1x10^4$ cells /well on 24-well plate (BD Falcon) coated with 0.1% porcine gelatin solution (Millipore, Billerica, MA) and cultured in hESF9(-/-) medium in the presence of varying growth factors. The cell numbers were counted by Coulter Counter (Beckman Coulter, Hialeah, FL).

Gene expression

A detailed reverse transcription-polymerase chain reaction (RT-PCR) protocol was described previously (Furue, *et al.*, 2005). Total RNA was extracted from hMSCs using RNeasy Mini Kit (Qiagen, Hilden, Germany) and SuperScript VILO cDNA Synthesis Kit (Invitrogen) according to the provider's instructions. Q-RT-PCR was carried out using the TaqMan gene expression Master Mix on in ABI PRISM 7300 Real-Time PCR system (Applied Biosystems, Foster City, CA) according to the supplier's instructions (ABI). Specific primers-probe set were listed in Supplementary Table 2. Expression levels were all normalized by the expression level of *GAPDH*. The relative level of each gene in cDNA of undifferentiated hES cells was defined as "1." The KhES-3 cell line was used as a control; the cells were obtained from the Institute for Frontier Medical Science, Kyoto University, and the Review Board of the National Institute of Biomedical Innovation approved this research.

Antigen expression

For *in situ* immunocytochemisty, the cells were immunostained with antibodies, as described previously (Draper *et al.*, 2002; Furue *et al.*, 2008). In this study, fluorescence images were acquired using by IN Cell Analyzer 2000 (GE Healthcare, Buckinghamshire, England). Flow cytometry was performed with BD FACS Canto flow cytometer (Becton Dickinson, San Jose, CA) as described previously (Draper *et al.*, 2002; Furue *et al.*, 2008). In this study, the labeled primary antibodies were used, but the biding of anti-SSEA-3, anti-CD56, and Tra-2-54 antibodies was visualized with RPE-conjugated goat anti-mouse Ig (Dako, Carpinteria, CA) or Alexa Fluor 647 goat anti-rat IgM (Invitrogen). The primary antibodies used are listed in Supplementary Table 3.

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投稿論文

ヒト多能性幹細胞の命名法の国際統一規格案について

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summary

In a few years, thousands of human embryonic stem (ES) / induced pluripotent stem (iPS) cell lines have been established in laboratories around the world. To date, confusions have arisen due to duplicate or redundant naming of cell lines. In addition, not all the important information such as provenance, derivation method and characterization are provided by researchers. To address these issues, a convention for naming and reporting human ES/iPS cell lines is urgently called. Recently Stem Cell Banks and researchers in the US, UK, China, Australia and the other countries proposed a new nomenclature system and a minimum set of criteria for reporting newly generated human ES/iPS cell lines. In this review, we have introduced their recommendations for developing a rule for naming and reporting of human ES/iPS cell lines.

はじめに

1998年にヒト胚性幹細胞(embryonic stem cell：ES細胞)[1)]が樹立され，2007年には，ヒト人工多能性幹細胞(induced pluripotent stem cell：iPS細胞)[2)]が開発された。これらの多能性幹細胞は，発生や疾患メカニズム解明など基礎研究のみならず，再生医療や創薬，毒性評価，ワクチン作製などへ応用の期待が高まっている。ヒトES/iPS細胞の株数は急ピッチで増加しており，すでに数千株にも及ぶ。実用化への研究を進めるために，国際幹細胞バンキングイニシアティブ(International Stem Cell Banking Initiative：ISCBI)では各国の細胞バンクや樹立機関が協力して世界中の研究者が相互に利用できる環境の整備を推進している。ところが，ヒトES/iPS細胞株の命名法について整備されておらず，混乱が生じている。このような現状から，2011年4月に，米国，英国，オーストラリア，中国などの幹細胞バンクや幹細胞研究者らから，「ヒトES/iPS細胞株の命名法および発表に関する標準化」[3)]が提案され，さらにその提案に対する意見[4)5)]が寄せられた。ISCBIや国際細胞バンク・ワーキンググループに参加する筆者らが，その内容を概説したい。

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ヒトES/iPS細胞の国際的な相互利用に向けて

2003年に設置された日本を含む22ヵ国からなる国際幹細胞フォーラム(http://www.stem-cell-forum.net/ISCF/)からの助成を受けて，2005年から英国シェフィールド大学Andrews教授が中心となって推進しているInternational Stem Cell Initiatives (ISCI)プロジェクトでは，日本(京都大学再生医科学研究所・中辻憲夫教授)を含めた世界11ヵ国のヒトES細胞樹立研究者らが連携して，ヒトES細胞株を登録し，樹立の方法，未分化/分化マーカーの発現などの解析方法とその結果を公表し[6)7)]，ヒトES細胞研究の標準化を進めてきた(http://www.stem-cell-forum.net/ISCF/initiatives/)。ISCIワークショップには筆者らも加わり標準化についての議論を行った。2008年からは，ヒトiPS細胞も含めて検討されている。さらに，ヒトES/iPS細胞株を各国間で相互に利用する体制を構築する必要があるとの認識のもとに，2007年から英国UK Stem cell Bankをはじめとする世界各国の細胞バンクが連携し，ISCBIプロジェクトが開始され，筆者らが参加している。このプロジェクトにおいては，ヒトES細胞のドナーの情報管理，資源化，品質管理法や分譲について，国際的にコンセンサスを図ってヒトES細胞を資源化するためのガイドラインを作成している[8)9)](和訳は，京都大学再生医科学研究所・細胞プロセシング・高田らより本誌Vol.10 No.4，p79-96，2011に掲載されているので参照されたい)。さらに，相互利用するためには不可欠な細胞登録における「細胞株の命名法」に関しても統一規定を設けることが現在の重要課題であり，国際的に活発な議論が展開されている。

これまでの現状

国内において細胞バンクが整備される1984年以前は，日本組織培養学会が細胞株を認定してJTCの番号を付与して登録する事業を実施していた。現在は，細胞バンクが整備され，JCRB(医薬基盤研究所細胞バンク，旧国立医薬品食品衛生研究所細胞バンク)，RCB(理化学研究所バイオリソースセンター細胞バンク)に，研究者が細胞株を寄託し，バンクの略称とともに登録番号で管理され，データベース上で公開されている。海外においても，米国のATCC，国立がん研究所(National Cancer Institute：NCI)，欧州細胞培養コレクション(European Collection of Animal Cell Cultures：ECACC)などの細胞バンクが各機関の略称や独自の登録番号(カタログ番号)を用いて管理し，情報を公開している。このように整備されていても，細胞株の情報や原著論文を検索するときに不都合が起こる。たとえば，“3T3 Swiss Albino”“3T3-Swiss albino”“Swiss-3T3”は同種の細胞株名であるが，データベースや論文での記載方法は他にも何通りも存在する。3T3と入力して検索すると，“3T3 (+3)”，“3T3-L1”，“3T3-SV40”など，別種の細胞株やサブクローンも検索にかかる。

まだ歴史の浅いヒトES/iPS細胞株においても，異なる研究機関で樹立された別個の細胞に全く同じ名前がつくといった問題がすでに生じている。たとえば，全く別の患者から採取した羊水(amniotic fluid：AF)に由来する2つのiPS細胞株の両方ともが“AF-iPS”と命名されたり[10)11)]，ジストロフィン遺伝子に異なる箇所に変異をもつ2人のデュシェンヌ型筋ジストロフィー (Duchenne muscular dystrophy：DMD)患者から樹立した全く別のiPS細胞であるにも関わらず，両方ともが“DMD-iPS1”と命名されたりしている[12)13)]。“iPS-1”や“iPS-WT”といった名称は汎用され，その名称のみから細胞株を特定することはできない[4)]。また，“KhES-1”“KhES-3”“HES-3”など，ヒトES細胞の名称に汎用される“HES”は，ヒト胎児皮膚(human embryonic skin：HES)由来線維芽細胞の株名“HES 5”[14)]などとも同じ表記であるため混同されやすい。細胞株を混同してしまえば，研究成果の妥当性，重要性

を正当に評価できなくなる。このように細胞命名法の国際的な統一規定がなかったことがデータベースの管理・利用を不便なものにしている。

ヒトES/iPS細胞の命名法の提案

2010年の国際幹細胞学会(International Society for Stem Cell Research：ISSCR, 2010年7月15日開催), およびISCI(2010年9月15日開催)のワークショップで議論された内容に準拠して，米国マサチューセッツ医科大学ヒト幹細胞バンクのInternational stem cell registry(ISCR)が代表として提案する「ヒトES/iPS細胞株の命名法および細胞登録に関する統一規定の案」が米国科学誌「Cell Stem Cell」2011年4月8日号[3]に掲載された。これに対し，京都大学iPS細胞研究所(CiRA)山中伸弥所長らの意見[4]と米国細胞バンクAmerican Type Culture Collection(ATCC) Brian Pollok所長らの意見[5]が同誌の6月3日号に掲載された。両者ともISCRの提案に大筋で同意した上で，幹細胞研究の将来展望をもとに想定される問題を提起し，改善案を提示した。

ヒトES/iPS細胞の命名法についての統一規定案

ISCRによる命名法の統一規定案(図)[3]は，特に次に示す5点に配慮したものである。①独自の識別方法(樹立機関IDと細胞株シリアル番号)を採用し，細胞株間で混同しないようにすること。②細胞株に関する情報が直感的に認識できること。③既存の細胞株名の表記方法(例：KhES-1, KhES3, CT4, B124-2)と同じフォーマットを採用すること。④異なる系統の細胞株であること(例：TSRI68iとSHEF4e-ALS)や，同じ系統の細胞株であること(例：SHEF3とSHEF5)を容易に認識できること。⑤柔軟性のあるルールにすること。

その細胞の名称の表記方法は図[3]に示すような4つの構成要素からなるものであり，(a)細胞株の樹立機関のID，(b)細胞株のシリアル番号(ID)，(c)ES細胞またはiPS細胞を区別する略号，および(d)細胞の特徴を示す情報を記載する。さらに，データベース上で処理するため，規定された場所にハイフンを使用する，文字数と数字の桁数を規定する，スペースを使用しない，ハイフンを含めて14桁に統一することを提案[3]している。

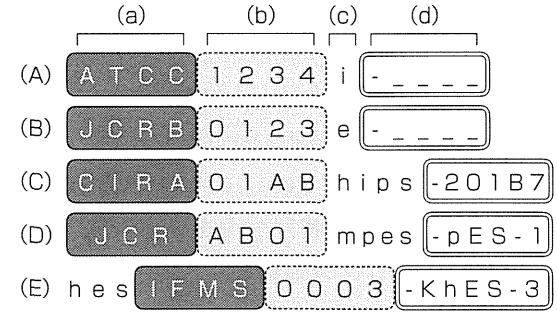

図1　ヒトES/iPS細胞株の命名法の案
(A)(B) ISCR[3]，(C)(D)山中教授ら[4]，(E)筆者らの案による細胞株名表記の例
(a)細胞株を樹立した研究機関(研究室または研究所)のID
(b)細胞株のID
(c)細胞種や由来を識別する記号。ISCRの提案[3]によると“iPS細胞”を“i”，“ES細胞”を“e”で表す。
(d)“-(ハイフン)”とその後に続くアルファベットまたは数字で細胞株の特徴やクローン番号等を表す。ISCR[3]は，(c)と(d)の部分は任意とし，(a)と(b)のみで細胞名を表すことも考慮している。それぞれの要素を表す部分に使用する文字数と数字の桁数に自由度をもたせることも可能だが，データベース管理及び検索の便宜上，①スペースを含まない，②アルファベットの大文字や小文字の表記法，数字の桁数，ハイフンの位置なども統一し，③全体で14文字に限定したものが望ましいとしている。細胞株名の表記法については，さらに議論が必要である。　(文献3より引用改変)

ヒトES/iPS細胞サブクローン株の数への対応

iPS細胞は1種類のドナー細胞から100種類以上のクローンを作製することもある。山中所長らは，細胞株を識別するためのIDの表記(図(b)の部分)は増大する株数に対応し得る方式でなければならないと提言[4]