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BRIEF REPORT

Registration of Human Embryonic Stem Cell Lines: Korea, 2010

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Abstract

In an effort to increase the credibility of human embryonic stem cell (hESC) lines established in Korea, obligatory registration was introduced by the Bioethics and Safety Act 2008, effective as of January 1, 2010.
The DNA fingerprint, chromosome stability, expression of pluripotency markers, and contamination of mycoplasma of the submitted lines were analyzed by Korea Centers for Disease Control and Prevention (KCDC). The characterization data and ethical aspects, such as informed consent for donation of surplus embryos, were reviewed by a 10-member advisory review committee for stem cell registry. A total of 55 domestic hESC lines were submitted for registration in 2010; among them 51 were registered. Among these submitted lines, 26 were additionally characterized by KCDC, while 25 lines previously characterized by the Ministry of Education, Science and Technology were not additionally analyzed by KCDC.
Registration completed an oversight system for embryo research by registering the products of licensed embryo research projects, making embryo research more transparent in Korea. Information about hESC lines is available at the website of the Korea Stem Cell Registry (kscr.nih.go.kr).

1. Introduction

A recent clinical trial on a stem cell therapy was initiated using derivatives from embryonic stem cells (ESCs) [1]. Human ESCs (hESCs) have a wide variety of possibilities in regenerative medicine, because of their two advantageous characteristics that distinguish them from adult stem cells: (1) embryonic stem cells can differentiate into any type of cell or tissue; and (2) embryonic stem cells have the capacity to proliferate indefinitely under optimal conditions. However, there are several obstacles in the use of hESCs that need to be overcome before they can be used in regenerative medicine. In addition to the scientific aspects, such as accurate control of proliferation and purity of differentiated cells, the reliability of the cell line is an ethical issue. This is because hESCs originate from human embryos. As the need for comprehensive information

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about the characteristics of hESC lines and provenance increased, several countries and groups established hESC registries [2,3]. The European registry, hESCreg (http://www.hescreg.eu/), was founded as a collaborative and interdisciplinary platform where researchers and the general public can access information about available hESC lines. The international stem cell registry at the University of Massachusetts Medical School (http://www.umassmed.edu/iscr/) has been continuously expanding its database since its launch in 2008, and currently has information on >400 hESCs and 120 induced pluripotent stem cells (iPSCs). In accordance with an order from the President of the United States. The National Institute of Health (NIH) has registered 91 hESC lines eligible for federal funding since 2009 (http://stemcells.nih.gov/research/registry). These registries provide information through their websites with features relevant to their own purposes.

Several scientists have been active in the production of hESC lines in Korea [4,5]. Approximately 60 hESC lines have been known to be established in Korea. Although the Bioethics and Safety Act has been effective since 2005, the exact information about hESC lines was not available. To improve the credibility of hESC lines that have been established and used in Korea, an obligatory registration was initiated on January 1, 2010 according to the Bioethics and Safety Act 2008. Here, we report the first 1-year results of the registration.

2. Materials and Methods

2.1. Cell culture

The hESC line H9 was cultured on STO (ATCC Manassas, VA, USA) feeder in 80% DMEM/F12 medium (Gibco, Carlsbad, CA, USA) supplemented with 20% KO serum (Gibco), 0.1 mM nonessential amino acids (Gibco), 0.1 mM β-mercaptoethanol (Gibco), and 4 ng/mL bFGF (Invitrogen). The STO feeder cells were maintained in DMEM medium containing 10% FBS (Gibco), and used after mitotic inactivation by mitomycin C (Sigma, St. Louis, MO, USA). H9 cells were passaged every 5−7 days with 2 μL/mL ROCK inhibitor (Sigma), and the culture medium was changed daily. The submitted hESC lines were minimally cultured in their own media. The human fibroblast cell line IMR-90 (ATCC) was maintained in DMEM medium containing 10% FBS and 1% penicillin/streptomycin (Gibco).

2.2. DNA fingerprinting

The identity of the hESC line was analyzed by short tandem repeat (STR) analysis. The hESC colonies were removed from the surface of the culture dish and collected in a 15-ml conical tube. The STR analyses were performed by DowGene (Seoul, Korea) and Kogene (Seoul, Korea); both companies used a Powerplex 16 system (Promega). The STR data of the submitted hESC lines were compared with those of other lines with clustering software R and Microsoft Excel.

2.3. Karyotyping

Karyotyping of hESCs was conducted by GenDix (Seoul, Korea) and Samkwang (Seoul, Korea). For karyotyping, a 4-well dish of each cell line was analyzed by GTG-banding method.

2.4. RT-PCR

Total RNA was isolated using Trizol (Invitrogen) according to the manufacturer's protocol. Subsequently, cDNA was synthesized from 1 μg of the total RNA by PrimeScript 1st strand cDNA synthesis kit (TAKARA). Quantitative real-time PCR (qRT-PCR) was performed with TaqMan assays (Applied Biosystems) and Gene Expression probes (Applied Biosystems; GAPDH, Hs99999905_m1; NANOG, Hs02387400_g1; OCT4, Hs00742896_s1; SOX2, Hs00602736_s1; TERT, Hs00162669_m1). qRT-PCR was performed using TaqMan Universal PCR Master Mix and ABI 7500 Real-Time PCR System (Applied Biosystems). Expression levels were analyzed by ΔΔCt method. All reactions were duplicated.

2.5. Mycoplasma tests

Two methods were used to estimate mycoplasma contamination in the hESCs. Tests were performed immediately after receiving cells from applicants. For PCR detection, PCR Mycoplasma Detection Set (TARAKA) was used with 0.5 μL ES culture media. For enzymatic detection, MycoAlert Mycoplasma Detection Kit (Lonza, Basel, Switzerland) was used with 100 μL ES culture media. Fresh ES culture media and positive control contained in the kit were used as controls. The amplified PCR products were analyzed by gel electrophoresis with 1% agarose gels. Enzymatic reactions were analyzed by Sirius L tube Luminometer (Berthold, Pforzheim, Germany).

3. Results

3.1. Procedure for registration

The registration procedure is shown in Figure 1. A stem cell scientist submitted application forms with copies of informed consent for use of surplus embryos. After a pre-review of the submitted files by the advisory review committee, we requested and analyzed the hESC lines. We analyzed DNA fingerprints, karyotypes, pluripotency markers, and mycoplasma contamination. The submitted files and our analyzed data were reviewed by the advisory review committee, which consisted of seven stem cell scientists, one bioethics professor, and two government employees. We had four committee

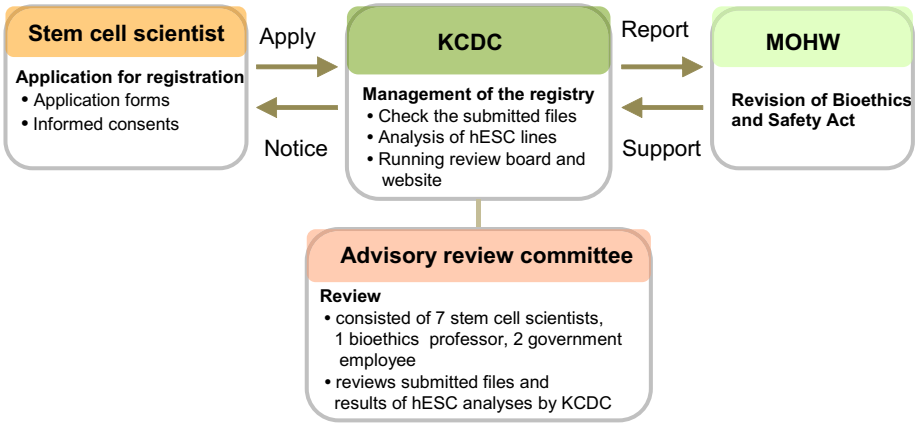

Figure 1. Registration procedure for hESC lines in Korea. KCDC runs the registry and the advisory committee reviews the submitted data for the registry.

meetings and two document reviews for the registration in 2010.

3.2. Informed consent

The Bioethics and Safety Act has been in effect since 2005. According to this act, only surplus embryos should be used for research. Therefore according to the provisions of this act, fertility clinics need to obtain informed consent from patients before the surplus embryos can be used for research. The registration indicated that 29 hESC lines were established from 917 surplus frozen embryos donated from 151 patients since 2005. It is now confirmed that all hESC lines were established from surplus embryos that have been provided by patients, with their informed consent, since 2005.

3.3. Analyses of hESC lines

Among 51 registered hESC lines, we analyzed the DNA fingerprint, karyotype, expression of pluripotency markers, and mycoplasma contamination of 27 lines. We harvested and analyzed hESCs as soon as the cells were submitted, but the cells were maintained for 1−2 days if the amount of cells available was insufficient for analyses. Another 25 lines were verified by the Ministry of Education, Science and Technology (MOEST) and were not additionally analyzed by us. A summary of the verified data is shown in Table. DNA fingerprinting using short tandem repeat (STR) analyses showed that all except analyzed lines except for one line are independent lines (Table). Among the 27 lines analyzed by us, three lines showed abnormal karyotypes (Figure 2A−D). Expression of pluripotency marker genes was detected in all the tested lines, although the expression levels of several pluripotency markers of an aneuploid hESC line (CHA-hES 16) were relatively lower than those of other hESC lines (Figure 2E). Mycoplasma contamination tests were performed twice if the first analysis yielded positive results. Eight lines were mycoplasma-positive in the first analysis and were resubmitted. Two lines were still mycoplasma-positive in the second analysis of submitted cells, while the other lines were negative in the second analysis of submitted cells, as shown in Table.

4. Discussion

The Korea hESC registry was founded to ensure that hESC research in Korea is ethically responsible and conducted in accordance with the Bioethics and Safety Act by providing reliable information about hESC lines. Our registry is enforced by the Bioethics and Safety Act 2008; under this act, registration of hESC lines is obligatory in Korea. The obligatory registration system is feasible in Korea because researchers who want to use human surplus embryos are required to obtain a license from the MOHW, and the hESC line is thus a product of licensed research. The Bioethics and Safety Act has been in effect since 2005, and obligatory registration started in 2010.

As a result of the registration, we confirmed that 29 hESC lines established after 2005 were from 917 surplus embryos obtained with informed consent. Among the 55 submitted lines, two lines could not be registered, and two other lines are under review. Dr. Hwang's Sooam-hES1 (also known as NT-1) line was ineligible for registration because of the unethical aspects revealed by the report of the investigation committee of Seoul National University [6]. In addition, NT-1 was revealed to be a product of parthenogenesis [6,7], which is not allowed under the Bioethics and Safety Act. Another hESC line that could not be registered is AMC-1, which showed the same DNA fingerprint as a previously established hESC line (Miz-hES4). When we requested the researchers to submit early passage of AMC-1, we were informed that it failed to grow. Among the 51 registered lines, 10 lines have abnormal karyotypes, as shown in Table. The hESC lines with abnormal karyotypes might be used as models of aneuploid chromosomal syndrome [8−10].

Table. Summary of analyses of hESC lines submitted in 2010

			Submitted data								KCDC-analyzed data					
[a]	Names of hESC lines[b]	Year	Karyotype	STR	Immuno-staining	RT-PCR	Mycoplasma	EB or teratoma	Copies of informed consents	List of surplus embryos	Karyotype	STR	Immuno-staining	RT-PCR	Mycoplasma	Registered
1	**SNUhES1**	2001	46, XY or 47, XY,+12	√	+	+	−	Teratoma				√[c]				Yes
2	**SNUhES2**	2001	46, XX	√	+	+	−	Teratoma				√[c]				Yes
3	**SNUhES3**	2001	46, XY	√	+	+	−	Teratoma				√[c]	+[c]	+[c]		Yes
4	**SNUhES4**	2003	46, XY	√	+	+	−	Teratoma				√[c]	+[c]	+[c]		Yes
5	**SNUhES5**	2004	47, XY,+16	√	+	+	−	Teratoma				√[c]				Yes
6	**SNUhES6**	2004	46, XY	√	+	+	−	EB				√[c]				Yes
7	**SNUhES7**	2004	46, XY	√	+	+	−	Teratoma				√[c]				Yes
8	**SNUhES10**	2004	69, XXX	√	+	+	−	EB				√[c]				Yes
9	**SNUhES11**	2004	46, XY	√	+	+	−	Teratoma				√[c]	+[c]	+[c]		Yes
10	**SNUhES12**	2004	46, XX	√	+	+	−	EB				√[c]				Yes
11	**SNUhES14**	2004	46, XY	√	+	+	−	EB				√[c]				Yes
12	**SNUhES16**	2004	46, XY	√	+	+	−	Teratoma				√[c]	+[c]	+[c]		Yes
13	**SNUhES18**	2004	46, XY or 47, XY,+12	√	+	+	−	Teratoma				√[c]				Yes
14	**SNUhES19**	2004	46, XY or 47, XY,+12	√	+	+	−	Teratoma				√[c]				Yes
15	**CHA-hES 3**	2005	46, XY	√	+	+	−	Teratoma	√	√		√[c]	+[c]	+[c]		Yes
16	**CHA-hES 4**	2004	46, XY	√	+	+	−	Teratoma	√	√		√[c]	+[c]	+[c]		Yes
17	**CHA-hES 5**	2004	46, XY, inv(9)	√	+	+	−	Teratoma	√	√		√[c]				Yes
18	**CHA-hES 6**	2004	46, XX	√	+	+	−	Teratoma	√	√		√[c]				Yes
19	**CHA-hES 7**	2004	46, XX	√	+	+	−	Teratoma	√	√		√[c]				Yes
20	**CHA-hES 8**	2004	46, XX	√	+	+	−	Teratoma	√	√		√[c]				Yes
21	**CHA-hES 9**	2004	46, XX	√	+	+	−	Teratoma	√	√		√[c]	+[c]	+[c]		Yes
22	**CHA-hES 10**	2005	46, XY	√	+	+	−	Teratoma	√	√		√[c]				Yes
23	**CHA-hES 11**	2005	46, XY	√	+	+	−	Teratoma	√	√		√[c]				Yes
24	**CHA-hES 12**	2005	46, XY	√	+	+	−	Teratoma	√	√		√[c]				Yes
25	**Miz-hES4**	2003	46, XY	√	+	+		EB				√[c]	+[c]	+[c]		Yes
26	**CHA-hES 13**	2007	46, XX	√	+	+	−	Teratoma	√	√	46, XX	√		+	−[d]	Yes
27	**CHA-hES 14**	2007	46, XY	√	+	+	−	Teratoma	√	√	46, XY	√		+	−[d]	Yes
28	**CHA-hES 15**	2007	46, XY	√	+	+	−	Teratoma	√	√	46, XY	√		+	−	Yes
29	**CHA-hES 16**	2007	71, XXY	√	+	+	−		√	√	70, XXY,+12	√		+	−[d]	Yes
30	**CHA-hES 17**	2007	46, XY	√	+	+	−	Teratoma	√	√	46, XY	√		+	−[d]	Yes
31	**CHA-hES 19**	2007	47, XX, der(2), -4,+5,+mar	√	+	+	−		√	√	47, XX, t(2;4) q(11.2;p15.2),+5	√		+	−[d]	Yes
32	**CHA-hES 20**	2007	46, XY	√	+	+	−	Teratoma	√	√	46, XY[e]	√		+	−	Yes
33	**CHA-hES 21**	2008	46, XX	√	+	+	−	Teratoma	√	√	46, XX	√		+	−	Yes

34	**CHA-hES 23**	2008	46, XX	√	+		−	Teratoma	√	√	46, XX	√	+	−	Yes
35	**CHA-hES 24**	2008	46, XY	√	+	+	−	Teratoma	√	√	46, XY	√	+	−	Yes
36	**CHA-hES 26**	2008	Tri- or tetraploidy	√	+		−		√	√	90, XXXX,-6, -18,add(20)	√	+	−	Yes
37	**SNUhES31**	2009	46, XY	√	+	+		EB	√	√	46, XY	√	+	−[d]	Yes
38	**CHA-hES 18**	2007	46, XX	√	+	+	−	Teratoma	√	√	46, XX	√	+	−	Yes
39	**CHA-hES 22**	2008	46, XX	√	+	+	−	Teratoma	√	√	46, XX	√	+	−	Yes
40	**CHA-hES 25**	2008	46, XX	√	+		−	Teratoma	√	√	46, XX	√	+	−	Yes
41	**CHA-hES M1**	2008	46, XX	√	+	+	−	Teratoma	√	√	46, XX	√	+	−	Yes
42	**CHA-hES R1**	2008	46, XY	√	+	+	−	Teratoma	√	√	46, XY	√	+	−	Yes
43	**CHA-hES R2**	2008	46, XX	√	+	+	−	Teratoma	√	√	46, XX	√	+	−	Yes
44	**CHA-hES R3**	2008	46, XX	√	+	+	−	Teratoma	√	√	46, XX	√	+	−	Yes
45	**CHA-hES R4**	2008	46, XY	√	+	+	−	Teratoma	√	√	46, XY	√	+	−	yes
46	**CHA-hES B1**	2009	46, XY	√	+	+	−	Teratoma	√	√	46, XY	√	+	−	Yes
47	**CHA-hES B2**	2009	46, XX	√	+	+	−	Teratoma	√	√	46, XX	√	+	−	Yes
48	**CHA-hES B3**	2009	46, XY	√	+	+	−	Teratoma	√	√	46, XY	√	+	−	Yes
49	AMC-1	2006	46, XY		+	+		Teratoma	√	√	46, XY	√[f]	+	−	No
50	**JNU-hES-01**	2009	46, XY	√	+	+		Dif[h]	√	√	46, XY	√	+	+[g]	Yes
51	**JNU-hES-02**	2009	47, XY,+12	√	+	+		Dif[h]	√	√	46, XY	√	+	+[g]	Yes
52	MB01	2002	46, XY		+	+									Under review
53	MB06	2004	46, XY		+	+									Under review
54	Sooam-hES1	2003	46, XX	√	+	+	−	Teratoma							No
55	**SNUhES32**	2010	46, XY	√	+	+	−	EB	√	√	46, XY	√	+	−	Yes

[a]Is the submitted number in the order of the review; [b]Registered lines are indicated with bold; [c]Analyzed by MOEST; [d]Mycoplasma-positive in analysis of first submitted cells, but negative in analysis of second submitted cells; [e]Abnormal karyotype in analysis of first submitted cells, but normal in analysis of second submitted cells; [f]Same STR with Miz-hES4; [g]Mycoplasma-positive in analyses of first and second submitted cells; [h]Differentiation data.

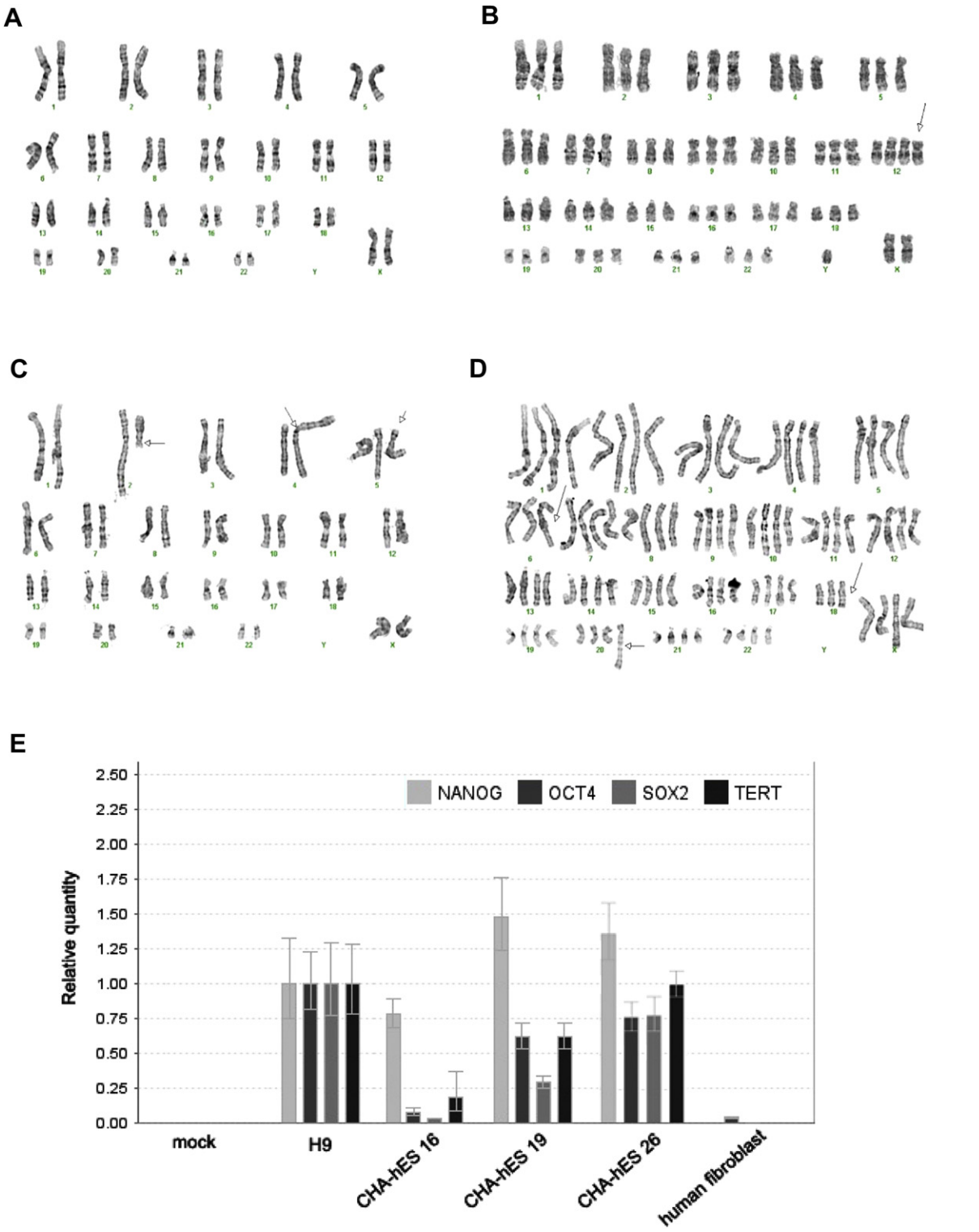

Figure 2. Characteristics of three hESC lines with abnormal karyotypes. (A) Normal karyotype of H9. (B) Abnormal karyotype (70, XXY,+12) of CHA-hES 16. (C) Abnormal karyotype (47, XX, t(2;4)(q11.2;p15.2),+5) of CHA-hES 19. (D) Abnormal karyotype (90, XXXX,-6,-18,add(20)) of CHA-hES 26. (E) The relative quantities of pluripotency marker genes were detected by real-time PCR using Taqman probe. CHA-hES 16 showed lower levels of expression of several pluripotency markers, whereas other abnormal lines showed normal expression patterns. H9 was used as a positive control; mock and human fibroblasts were used as negative controls.

Recently, methods for establishing an iPSC line were developed [11,12]. iPSCs have characteristics similar to hESCs with respect to their pluripotency and self-renewal capacity. Human iPSCs have been recognized as a good model system for genetic diseases and drug screening [13]. We are considering the registration of human iPSC lines to provide information to the scientific community using the hESC registration system. The registration of human iPSC lines will be optional, not obligatory. We hope that this registry (kscr.nih.go.kr) promotes transparency of stem cell research in Korea.

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