Materials Submitted to NIH
from the Third Affiliated Hospital of Guangzhou Medical College
Submission #2010-ACD-002

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NOTE: Duplicative information in the submission is not included. *In particular, ten (10) copies of the embryo donation consent sent by submitter with 2007 donation dates. One copy of the translated consents is included here for simplicity.

(1)

hESC Registry Application Database

Detailed Listing for Request #: 2010-ACD-002
November 26, 2010

hESC Registry Application Search Results

Request #: 2010-ACD-002
Status: Pending

Review: ACD

Assurance: Yes (Section II(B))
Certification: Yes
Authority: Yes

Cell Lines: 6
Available: 6

Previous #:
2010-DRAFT-006

Email
Edit
Delete
Switch to ADM

Organization: THE THIRD AFFILIATED HOSPITAL OF GUANGZHOU MEDICAL COLLEGE
Org Address: No.63, Duobao Road, Guangzhou , Guangdong , China
DUNS: 527189518 **Grant Number(s):**
Signing Official (SO): THE THIRD AFFILIATED HOSPITAL OF GUANGZHOU MEDICAL COLLEGE / 862081292013 / xiaofangsun@hotmail.com
Submitter of Request: xiaofangsun / 862081292013 / xiaofangsun@hotmail.com
Submitter Comments: The Informed Consent to Donate Discarded Embryos are Chinese, we translate them into English. If there are problem or unclear ,please contact us.

Line #1: FY-hES-1
NIH Approval #:
Available: Yes
Embryo from U.S.: No
Embryo Donated in Year(s):
Provider Name: THE THIRD AFFILIATED HOSPITAL OF GUANGZHOU MEDICAL COLLEGE
Provider Phone: 862081292013
Provider Email: xiaofangsun@hotmail.com
Provider URL:
Provider Restrictions: None

NIH Restrictions:

Additional Information:

Line #2: FY-hES-3
NIH Approval #:
Available: Yes
Embryo from U.S.: No
Embryo Donated in Year(s):
Provider Name: THE THIRD AFFILIATED HOSPITAL OF GUANGZHOU MEDICAL COLLEGE
Provider Phone: 862081292013
Provider Email: xiaofangsun@hotmail.com
Provider URL:
Provider Restrictions: None

NIH Restrictions:

Additional Information:

Line #3: FY-hES-5
NIH Approval #:

(2)

Available: Yes
Embryo from U.S.: No
Embryo Donated in Year(s):
Provider Name: THE THIRD AFFILIATED HOSPITAL OF GUANGZHOU MEDICAL COLLEGE
Provider Phone: 862081292013
Provider Email: xiaofangsun@hotmail.com
Provider URL:
Provider Restrictions: None

NIH Restrictions:

Additional Information: Karyotype is 46,XX, +13, der(13;13)(q10;q10)

Line #4: FY-hES-7
NIH Approval #:
Available: Yes
Embryo from U.S.: No
Embryo Donated in Year(s):
Provider Name: THE THIRD AFFILIATED HOSPITAL OF GUANGZHOU MEDICAL COLLEGE
Provider Phone: 862081292013
Provider Email: xiaofangsun@hotmail.com
Provider URL:
Provider Restrictions: None

NIH Restrictions:

Additional Information:

Line #5: FY-hES-8
NIH Approval #:
Available: Yes
Embryo from U.S.: No
Embryo Donated in Year(s):
Provider Name: THE THIRD AFFILIATED HOSPITAL OF GUANGZHOU MEDICAL COLLEGE
Provider Phone: 862081292013
Provider Email: xiaofangsun@hotmail.com
Provider URL:
Provider Restrictions: None

NIH Restrictions:

Additional Information:

Line #6: FY-3PN
NIH Approval #:
Available: Yes
Embryo from U.S.: No
Embryo Donated in Year(s):
Provider Name: THE THIRD AFFILIATED HOSPITAL OF GUANGZHOU MEDICAL COLLEGE
Provider Phone: 862081292013

Provider Email: xiaofangsun@hotmail.com
Provider URL:
Provider Restrictions: None

NIH Restrictions:

Additional Information: Karyotype is 69,XXX

Supporting Documents:
Document 1: (PDF - 02/26/2010) Markers used to characterise cell lines
Document 2: (PDF - 02/25/2010) SO letter
Document 3: (PDF - 02/26/2010) Informed Consent to Donate Discarded Embryos

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Administrative Comments: Replaced original informed consent document with one that redacted names of donor (husband and wife) and put document in pdf format 02-26-2010 by Betsy Dean

Uploaded Assurance for Section IIB - 25 May 2010 by DHannemann

Uploaded Donor Consent Form w/names redacted (Chinese) - 25 May 2010 by DHannemann

Uploaded IVF Clinical Treatment w/names redacted (Chinese) - 25 May 2010 by DHannemann

Uploaded Research Protocol (Chinese) - 25 May 2010 by DHannemann

Uploaded Ethics Committee Approval (Chinese) - 25 May 2010 by DHannemann

Uploaded Publication - 25 May 2010 by DHannemann

Uploaded Ethics Committee Approval (English) - 16 June 2010 by DHannemann

Uploaded IVF Clinical Consent (redacted-English) - 16 Jun 2010 by DHannemann

Uploaded Donor Consent (redacted-English) - 16 Jun 2010 by DHannemann

Uploaded Research Protocol (English) - 16 Jun 2010 by DHannemann

Uploaded NIH Staff IIB Analysis - 17 Jun 2010 by DHannemann

Uploaded Email Correspondence (30 Jun 2010) - 1 July 2010 by DHannemann

Exchanged original Donor Consent Form in Chinese with redacted version - 8 July by DHannemann

Exchanged original IVF Consent Form in Chinese with redacted version - 8

July by DHannemann

July 8 email response uploaded by E. Gadbois

July 8 SO certifications corrected E. Gadbois

July 11 email response uploaded by D. Hannemann on July 12 2010

July 21 submitter response email uploaded by D. Hannemann on July 22 2010

July 22 submitter response email uploaded by D. Hannemannon July 27 2010

July 26 submitter response email uploaded by D. Hannemann July 27 2010

Compilation of submission documents uploaded by D. Hannemann 18 Nov 2010

Availability of line FY-3PN corrected per clarification from submitter and karyotypes added, E. Gadbois 19 Nov 2010

Administrative Attachments:

Document 1: (PDF - 05/25/2010) Assurance for Section IIB
Document 2: (PDF - 07/08/2010) Donor Consent Form (Chinese) - redacted
Document 3: (PDF - 07/08/2010) IVF Clinical Treatment Consent (Chinese) - redacted
Document 4: (PDF - 05/25/2010) Research Protocol (Chinese)
Document 5: (PDF - 05/25/2010) Ethics Committee Approval (Chinese)
Document 6: (PDF - 05/25/2010) Publication
Document 7: (DOC - 06/16/2010) Ethics Committee Approval - English
Document 8: (PDF - 06/16/2010) IVF Clinical Consent - English - Redacted
Document 9: (PDF - 06/16/2010) Donor Consent - English - Redacted
Document 10: (PDF - 06/16/2010) Research Protocol - English
Document 11: (DOC - 06/17/2010) NIH Staff IIB Analysis
Document 12: (PDF - 07/01/2010) Email Correspondence (30 June 2010)

Document 13: (PDF - 07/08/2010) July 8 response from Guangzhou
Document 14: (PDF - 07/12/2010) Submitter response email July 11, 2010
Document 15: (PDF - 07/22/2010) 21 July 2010 Submitter Response Email
Document 16: (PDF - 07/27/2010) 22 July 2010 Submitter Response Email
Document 17: (PDF - 07/27/2010) 26 July 200 Submitter Response Email

Document 18: (PDF - 11/19/2010) Compilation of submission documents (as of 18 Nov 2010)
Document 19: (PDF - 11/22/2010) Guangzhou response Nov 16 2010

Status History:
Draft: 02/25/2010

(5)

Assurance

Date :May 18, 2010

NIH Stem Cell Registry:

I hereby assure that the embryo from which the cell line(s) identified in item 6 of the form was derived was donated prior to July 7, 2009, and the embryo:

1) was created using in vitro fertilization for reproductive purposes and was no longer needed for this purpose; and

2) was donated by individuals who sought reproductive treatment ("donor(s)") who gave voluntary written consent for the human embryo to be used for research purposes.

The applicant is advised that the Working Group of the Advisory Committee to the NIH Director will consider submit ted materials taking into account the principles articulated in Section II(A) of the NIH Guidelines for Human for Human Stem Cell Research, 45 CFR 46 Subpart A, and the following points to consider: during the informed consent process, including written and oral communications, whether the donor(s) were:

(1) informed of other available options pertaining to the use of the embryo ;

(2) offered any inducements for the donation of the embryo ; and

(3) informed about what would happen to the embryo after the donation for research."

Name and signature : Xiaofang Sun

Informed Consent to Donate Discarded Embryos

Research Project : Establishment of human stem cell bank

Research Unit : Guangzhou Key Laboratory of Reproductive and Genetics, The Third Affiliated Hospital of Guangzhou Medical College, Duobao Road 63#, 510150 ,Guangzhou, China

If you do not understand the terminology, please ask the physician or working group members to explanation of any terms or information that you do not know.

Research Objective: Derivation of human embryonic stem cell lines

Privacy : Your medical records and the signed written consent will be kept by Guangzhou Key Laboratory of Reproductive and Genetics, The Third Affiliated Hospital of Guangzhou Medical College. The research results may be published in academic conferences or journals, but your name will be never appeared in the published data.

Benefits: Specimens provided by you will be of great significance to the establishment of human embryonic stem cell bank and the development of stem cell research.
Donation is voluntary behavior, you will not receive any benefit in this trial.

Questions: If you have any questions , please contact with Xiaofang Sun, 020-81292202.

Donors pledge : We have read and understand the information about the research project. We have clearly known the basic concepts of embryonic stem cells. we have known that the study does not produce a new individual or the descendants. The ethical and legal issues involved in the research project have already been made a comprehensive explain. We have got a satisfactory answer that we asked about our participation . Based on personal preference, we are independent, voluntary, not subject to any conditions, threats and forced to sign this consent. We agreed to provide the discarded embryos and medical records tothe Third Affiliated Hospital of Guangzhou

Medical College.

We know that it is not a commercial activity of participating in the research. We just provided the discarded embryos in the research project. We will not get any benefits.

We can not track the progress and results of the research project. We have no direct relationship with the research results.

The discarded embryos can not be available to any individual or research units without our consent.

The discarded embryos can not be used to other experiment without our consent.

After signing this consent , we confirm that we agree to provide the discarded embryos for the research project, while we do know that we will not be injuryed to any legal rights.

A total of two pages of this consent, in duplicate, one kept by the donor, the other kept by the research group.

Wife: (Signature) **Husband:** Signature)

Date: March 5,2007 **Date:** March 5,2007

Deputy of The Third Affiliated Hospital of Guangzhou Medical College :Xiaofang Sun(Signature)

Date: March 5,2007

INFORMED CONSENT ON DONATION OF DISCARDED EMBRYOS

TEST TITLE: Establishment of a Human Embryonic Stem Cell Bank

RESEARCH INSTITUTIONS: Guangzhou City Key Laboratory on Reproduction and Genetics
The Third Hospital Affiliated to Guangzhou Medical University
63 Duobao Road, Liwan District, Guangzhou, China 510150

This Informed Consent form may contain certain technical terms that you do not understand. You may ask the doctor in charge of the test or members of the test team to explain any technical terms or information that you do not understand.

Object of the Test: To establish a human embryonic stem cell bank for the purposes of scientific research only but not for clinical use.

Privacy and Confidentiality

Your medical record and the informed consent that you sign will be stored as classified documents and record by the Guangzhou City Key Laboratory on Reproduction and Genetics, the Third Hospital Affiliated to Guangzhou Medical University. The result of the test may be published at academic conferences or on academic journals, but your name, telephone number and other information unrelated to the content of the research will never appear in the publications.

Benefits

The specimens you provide will be of tremendous significance to the establishment of human embryonic stem cell banks. The embryonic stem cell lines so established will be stored for an extended period of time, will be provided to scientific researchers in their studies, and may have commercial value in the future. However, you will not benefit from this test.

Questions

If you have other questions about participation in this study, please contact the following individual: Sun Xiaofang, 020-81292202.

The Subject's Acknowledgement

We have read and understood the above information about this test, are clear about the concept of embryonic stem cell, and know that the study will not produce a new individual or offspring. The person in charge has provided a full explanation of the ethical and legal issues involved in the test, and given us an opportunity to ask questions about this test and our participation. We have received answers to our satisfaction. We have signed this informed consent based on our personal wishes, independently and voluntarily, and free from any duress or coercion, agreeing to provide the Third Hospital Affiliated to Guangzhou Medical University with test specimens and medical record.

We know that participating in this study is not a commercial act, that we merely provide unusable embryos discarded after an embryo transfer, and that we will not benefit from it.

We may not follow the specific progress and result of the test, and the outcome of the test bears no direct relationship with us.

The specimens may not be provided to any individual or research institution without our consent.

The specimens may not be used in any other test in the laboratory without our consent.

By signing this informed consent, we confirm that we agree to provide specimens for this test. Meanwhile, we have made sure that we will not compromise any legal right by participating in this test.

This consent contains two pages in duplicate. One copy is to be kept by the donor of the embryo, and the other will be archived by the test project team.

Wife's signature:
Date: March 5, 2007

Husband's signature:
Date: March 5, 2007

The Third Hospital Affiliated to Guangzhou Medical University
Representative's signature: Sun Xiaofang
Date: March 5, 2007

(10)

Department of Reproduction and Assisted Reproduction
Guangzhou City Institute of Gynecology and Obstetrics
The Third Hospital Affiliated to Guangzhou Medical University

INFORMED CONSENT ON IN VITRO FERTILIZATION AND EMBRYO TRANSFER

Dept. No.

We (wife): _____ and (husband): _____ [illegible] are legally married husband and wife. We authorize diagnosis and treatment of our infertility by the Department of Reproduction and Assisted Reproduction, Guangzhou City Institute of Gynecology and Obstetrics, the Third Hospital Affiliated to Guangzhou Medical University. The doctors have introduced to us the indications of IVF-ET, such as 1. The woman's gamete transportation disorder caused by a variety of factors; 2. Ovulation disorder; 3. Endometrial ectopia; 4. The man's inadequate or weak sperms; 5. Infertility for unknown reasons; 6. The woman's immunological infertility; or 7. _____. Our infertility is the fourth of the above indications.

The doctors recommended IVF-ET treatment in light of our condition. In addition, other methods of treatment were chosen as well, such as _____ [illegible].

After careful consideration, we have voluntarily elected IVF-ET.

We have been informed that IVF-ET as a treatment method does not guarantee a completely successful pregnancy. In consistency with our age and the causes for the infertility, the current clinical pregnancy rate is 30-40%. Last year's clinical pregnancy rate in the department was 30%.

The doctors have introduced to us the treatment process of IVF-ET, including routine pre-operation tests, drug-induced ovulation, B-ultrasound monitoring of the follicular development, vaginal egg retrieval under ultrasonic guidance, collection and processing of semen, in vitro fertilization, embryo incubation, embryo transfer and post-transfer drug support of corpus luteum, timely urinoscopy or blood test, and B-ultrasound monitoring of the embryonic growth and development.

The doctors also have made it clear to us that, during the process of treatment, the following adverse reactions and side effects, and sometimes even certain serious complications, may occur, possibly leading to the failure of the treatment. The doctors also have explained to us the preventive and treatment measures against these side effects, which may cause an increase of the cost of treatment. We understand.

1. Over-stimulation of the ovary. In serious cases, there can be nausea, abdominal pain, ascites, hemoconcentration, oliguria; in rare, severe cases, there can be a formation of thrombus or damage of the hepatic and renal functions, which can be life-threatening. Once these conditions occur, treatment can be made with drugs or paracentesis to draw off pleural and ascitic fluid.

2. Anesthetic accidents, damage to other organs or abdominal hemorrhage can occur during an egg-retrieval operation. An operation may be necessary for treatment.

3. Adverse reaction of the ovary: It will be necessary to adjust the drug dosage or even abandon the current cycle of treatment.

4. The operations of egg retrieval and transfer may cause infections and necessitate anti-infection treatment.

The following conditions may occur during the process of embryo incubation and transfer:

1. The treatment may need to be terminated due to failure to retrieve eggs during follicular puncture. In the event of failure to collect the semen, we agree [checked]/do not agree to freeze the eggs.

2. Abnormality of the sperms or eggs may result in the failure of the fertilization or cause embryos to stop developing, making embryo transfer impossible.

3. In the event of poor quality of the embryos during the incubation process, we agree [checked]/do not agree to abandon the transfer.

4. Since 2-3 embryos can be transferred during each IVF-ET operation, multifetal pregnancy is common. In the case of pregnancy with more than two fetuses, multifetal pregnancy reduction is necessary. We understand that a multifetal pregnancy reduction operation may result in miscarriage, hemorrhage, infection, and the need for more than one operation in the event of failure. We further understand that, given the current level of medical technology, doctors can only eliminate the embryos that are smaller in size and at the location where operation is easier, and that they cannot guarantee that the remaining embryos are free from malformation.

We are aware that there is no significant difference in the fetal malformation occurrence rate between the cases where this technology is used and the cases of natural pregnancy, and that therefore there is no guarantee that each child is born healthy. Furthermore, as in the cases of natural pregnancy, related pregnancy and delivery complications, such as miscarriage, ectopic pregnancy and hydatidiform mole, can occur where IVF-ET is used. Sometimes operations are necessary.

The doctors have explained to us that the cost of each cycle of IVF-ET treatment is approximately 15,000-30,000 yuan, regardless of whether the operation is successful. In the event that the treatment is terminated for any reason, we will be charged for the tests and treatment that have been completed.

We are aware that we have the right to select the way our gametes and embryos are handled, but that sale of the gametes and embryos is prohibited; that we have the right to request termination of the application of the technology at any time; and that doing so will not affect our treatment at the Department of Reproduction and Assisted Reproduction in the future. In order to ensure a normal pregnancy and the health of our children after birth, we will cooperate with the Department of Reproduction and Assisted Reproduction in the follow-up on our pregnancy and the children after birth, and provide the department with our detailed personal information such as mailing address and telephone numbers. We will provide the Department of Reproduction and Assisted Reproduction with the originals of each of our identification cards, certificate of marriage and reproduction permit and their copies, in compliance with the State laws and regulations on population and family planning.

We are certain that the sperms and eggs to be used in the process of this IVF-ET treatment were all obtained from us, and that the children born are completely our own, both genetically and legally.

We have the ethical, moral and legal right to and duty for the children born through IVF-ET treatment, including those with birth defects. They enjoy the same legal rights and obligations as the children born naturally, including the rights to inheritance and education, the duty to support their parents, and the right to custody in the event of divorce of their parents.

We are aware that the Department of Reproduction and Assisted Reproduction will keep confidential the information concerning the tests and treatment that we have undergone here, and that our consent is required for the disclosure of our personal information.

We have carefully read and completely understood the detailed rules and informed consent pertaining to the IVF-ET treatment, discussed our concerns with the doctors, and received answers to our satisfaction. We voluntarily elect IVF-ET as the method of our treatment, and have signed this informed consent.

Husband (signature): _____ [illegible] Date: September 28, 2007
Wife (signature): _____ Date: September 28, 2007
Doctor (signature): _____ [illegible] Date: September 28, 2007

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Department of Reproduction and Assisted Reproduction
Guangzhou City Institute of Gynecology and Obstetrics
The Third Hospital Affiliated to Guangzhou Medical University

INFORMED CONSENT ON INTRACYTOPLASMIC SPERM INJECTION (ICSI)

Dept. No. 9848

We are legally married husband and wife. We authorize diagnosis and treatment of our infertility by the Department of Reproduction and Assisted Reproduction, Guangzhou City Institute of Gynecology and Obstetrics, the Third Hospital Affiliated to Guangzhou Medical University. The doctors have introduced to us the indications of ICSI, such as 1. Serious inadequacy, weakness or malformation of sperms; 2. Irreversible obstructive azoospermia; 3. Spermatogenic disorder (excluding the condition caused by genetic defects); 4. Failure of in vitro fertilization; 5. Male immunological infertility; 6. Spermic acrosome abnormality; 7. Where preimplantation genetic test of embryos is necessary; and 8._____. The cause of our infertility is the first indication above.

The doctors recommended ICSI treatment in light of our condition. In addition, other methods of treatment, such as artificial insemination by donor, were available as well. After careful consideration, we have voluntarily elected the ICSI treatment method.

The doctors have informed us that ICSI as a means of treatment does not guarantee a completely successful pregnancy. In consistency with our age and the causes for the infertility, the current clinical pregnancy rate among those who selected this treatment is 30-40%. Last year's clinical pregnancy rate in the department was 30%.

The doctors have introduced to us the treatment process of ICSI, including routine pre-operation tests, drug-induced ovulation, B-ultrasound monitoring of the follicular development, vaginal egg retrieval under ultrasonic guidance, collection and processing of semen, fertilization by direct injection of a single sperm into an oocyte through a microinjector so that the oocyte will develop into an embryo, which is then transferred into the uterine cavity, drug-support of corpus luteum, timely blood test, and B-ultrasound monitoring of the embryonic growth and development.

We also have learned that, if there were insufficient sperms to perform ICSI on the day of the egg retrieval, we agree [checked]/do not agree to freeze the eggs.

We have learned that the current technology is safe in most cases, but as with IVF-ET, there is a risk of side effects and complications. However, the technology also comes with other risks. For example, microinjection may cause unknown damage to an egg; even if it is tested normal, the male chromosome may still transmit pathogenic genes that we carry to the next generation during this process. There is no significant difference in the fetal malformation occurrence rate between the cases where this technology is used and the cases of natural pregnancy, and there is no guarantee that each "test tube baby" will be born healthy.

We know we can give up or withdraw from the treatment at any stage, which will not affect our continued treatment at this medical facility in the future.

The doctors have explained to us that the cost of each ICSI cycle of treatment is approximately 4,000 yuan higher than the conventional IVF-ET, and that the cost is the same regardless of whether the treatment is successful. In the event that the treatment is terminated for any reason, we will be charged for the tests and treatment that have been completed.

We have carefully read and completely understood the detailed rules and informed consent pertaining to the ICSI treatment, discussed our concerns with the doctors, and received answers to our satisfaction. We voluntarily elect ICSI as the method of our treatment, and have signed this informed consent.

Husband (signature): _____ [illegible] Date: September 28, 2007
Wife (signature): _____ Date: September 28, 2007
Doctor (signature): _____ [illegible] Date: September 28, 2007

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Department of Reproduction and Assisted Reproduction
Guangzhou City Institute of Gynecology and Obstetrics
The Third Hospital Affiliated to Guangzhou Medical University

INFORMED CONSENT ON EMBRYO FREEZING AND THAWING

Dept. No._____

We have received assisted reproduction treatment at Guangzhou City Institute of Gynecology and Obstetrics, the Third Hospital Affiliated to Guangzhou Medical University. We request that the staff of the Department of Reproduction and Assisted Reproduction to cryopreserve the usable embryos left after an embryo transfer.

We understand that the purpose of the cryopreservation is to achieve pregnancy in a future treatment cycle by reviving embryos through the transfer process instead of inducing ovulation. This will not only save costs, but also maximize the use of embryos in order to raise the cumulative pregnancy rate for each induced ovulation treatment.

According to the doctors of the Department of Reproduction and Assisted Reproduction, embryos' tolerance to freezing and revival ability vary depending on their quality. Therefore, there may not be any transferable embryos due to cold injury. The hospital will nevertheless charge fees for cryopreservation, storage and thawing. We are fully prepared psychologically. We have learned that the embryo transfer rate after thawing is approximately 70-80% in the department, and that the post-transfer pregnancy rate in the department is approximately ____% [original is cut off].

In order to prevent multifetal pregnancy, we have learned and agreed that not more than three embryos should be transferred within each cycle under the Standards for the Assisted Reproduction Technology of the Ministry of Public Health, and that not more than two embryos among women under the age of 35 should be transferred in their first assisted reproduction cycle.

We are also aware that, at the current level of technology, there is no guarantee that each "test tube baby" as a result of the transfer of a frozen and thawed egg will be born healthy, and that, after pregnancy starts, there can be miscarriage, ectopic pregnancy, premature delivery, fetal malformation and other pregnancy and delivery complications.

We know that embryos cannot be preserved indefinitely, that the first paid storage period is one year after the freezing of the embryo, renewable each year, and that our ownership rights to the embryos are deemed relinquished if the renewal fee is not paid upon expiration of the storage period. We agree to allow the embryos to be:

1. discarded; or
2. used for educational and scientific research purposes after the labels are removed

We have discussed our concerns with the doctors, and have received answers to our satisfaction. We have signed this informed consent after being fully informed.

Husband (signature): _____ [illegible] Date: September 28, 2007
Wife (signature): _____ Date: September 28, 2007
Doctor (signature): _____ [illegible] Date: September 28, 2007

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Department of Reproduction and Assisted Reproduction
Guangzhou City Institute of Gynecology and Obstetrics
The Third Hospital Affiliated to Guangzhou Medical University

INFORMED CONSENT ON EGG RETRIEVAL THROUGH TRANSVAGINAL PUNCTURE UNDER INTRAVENOUS ANESTHESIA

Dept. No._____

I request egg retrieval through transvaginal puncture under intravenous anesthesia. The doctors have explained, and I understand, the following possible complications associated with anesthesia:

Possible complications associated with anesthesia;

1. Anesthetic accident
2. Allergy to anesthetics
3. Respiratory and circulatory inhibition

Note: Abstinence from food and liquid is necessary within six hours before operation.

Patient (signature): _____ Date: September 28, 2007
Doctor (signature): _____ [illegible] Date: September 28, 2007

****

ANESTHETIC RECORD

The patient underwent egg retrieval under intravenous anesthesia on October 29, 2007. Oxygen was inhaled, and various vital signs were monitored. BP 126/68 mmHg, R 18 times/minute, HR 82 times/minute, SPO_2 98%. Her condition was stable. She regained consciousness five minutes after operation, and was escorted out of the operation room after no discomfort was observed.

Drug: Propofol/Other _____ Dosage: 200 mg

Remark: _____

_____

Anesthetist (signature): _____ Date: October 29, 2007

(17)

The Third Hospital Affiliated to
Guangzhou Medical University

Upon review by the Institutional Review Board of this hospital, the study on the establishment of human embryonic stem cell lines with the discarded embryos donated by couples who have undergone infertility treatment through in vitro fertilization and embryo transfer conducted by the project team of Comrade Sun Xiaofang has been found to comply with the pertinent regulations in the State Ethnical Guidelines for Research on Human Embryonic Stem Cells. The IRB of this hospital, upon review, approves its research on human embryonic stem cell lines.

Institutional Review Board of the Third Hospital
Affiliated to Guangzhou Medical University

Xu Xuehu [signature]

Chairman

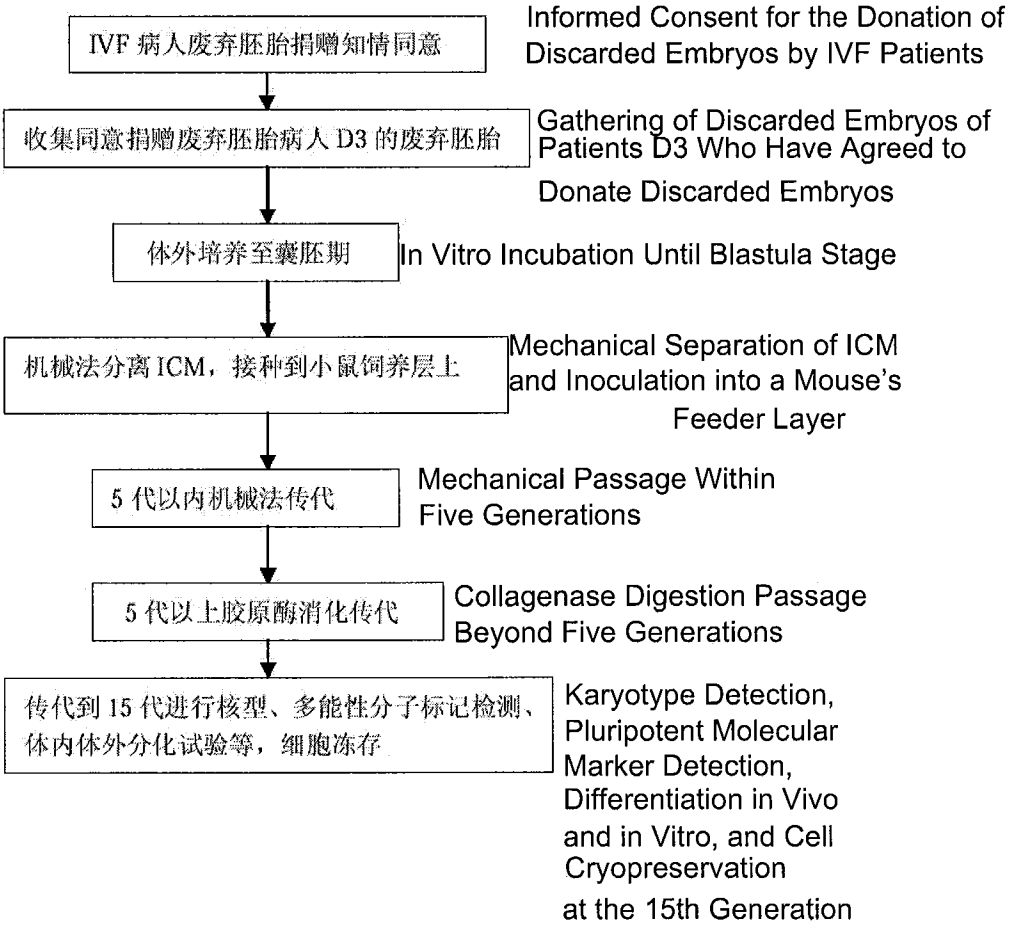
October 30, 2006

The Third Hospital Affiliated with
Guangzhou Medical University
Address: 63 Duobao Road, Liwan District, Guangzhou
Postal Code: 510150
Telephone: 020-81292288 81292023
Fax: 020-81292949

广州医学院第三附属医院

人胚胎干细胞建系流程

The Third Hospital Affiliated to Guanzhou Medical University
Flow Chart of Establishment of Human Embryonic Stem Cell Lines



Human Reproduction Vol.23, No.10 pp. 2185–2193, 2008
Advance Access publication on July 8, 2008
doi:10.1093/humrep/den137

Similar biological characteristics of human embryonic stem cell lines with normal and abnormal karyotypes

Xiaofang Sun[1], Xiaolin Long, Yifei Yin, Yonghua Jiang, Xinjie Chen, Weiqiang Liu, Wenhong Zhang, Hongzi Du, Shaoying Li, Yuhong Zheng, Shu Kong, Qianying Pang, Yu Shi, Yulin Huang, Shengchan Huang, Baoping Liao, Guohong Xiao and Weihua Wang

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BACKGROUND: Human embryonic stem cell (hESC) lines derived from poor quality embryos usually have either normal or abnormal karyotypes. However, it is still unclear whether their biological characteristics are similar. METHODS: Seven new hESC lines were established using discarded embryos. Five cell lines had normal karyotype, one was with an unbalanced Robertsonian translocation and one had a triploid karyotype. Their biological characteristics, short tandem repeat loci, HLA typing, differentiation capability and imprinted gene, DNA methylation and X chromosome inactivation status were compared between different cell lines. RESULTS: All seven hESC lines had similar biological characteristics regardless of karyotype (five normal and two abnormal), such as expression of stage-specific embryonic antigen (SSEA)-4, tumor-rejection antigen (TRA)-1-81 and TRA-1-60 proteins, transcription factor octamer binding protein 4 mRNA, no detectable expression of SSEA-1 protein and high levels of alkaline phosphatase activity. All cell lines were able to undergo differentiation. Imprinted gene expression and DNA methylation were also similar among these cell lines. Non-random X chromosome inactivation patterns were found in XX cell lines. CONCLUSIONS: The present results suggest that hESC lines with abnormal karyotype are also useful experimental materials for cell therapy, developmental biology and genetic research.

Keywords: human embryonic stem cell lines; characterization; karyotype; methylation; X-inactivation

Introduction

Human embryonic stem cell (hESC) research is one of the most rapidly growing areas in cell biology and medicine. Recent evidence has indicated that hESC can be cultured in the laboratory, unlimitedly passed from generation to generation (Thomson *et al.*, 1998; Stojkovic *et al.*, 2004; Oh *et al.*, 2005; Peura *et al.*, 2007) and induced to differentiate into all kinds of somatic cells under appropriate conditions. These differentiated cells can be used to restore damaged tissues and to treat some kinds of diseases (Assady *et al.*, 2001; Kehat *et al.*, 2001; Wang *et al.*, 2005; Lim *et al.*, 2006).

Since the first hESC line was established in 1998 (Thomson *et al.*, 1998), more than 400 hESC lines have been established in 20 countries and some of them have been registered in the National Institutes of Health (http:escr.nih.gov/) (Guhr *et al.*, 2006). To establish new hESC lines, human embryos are required. However, it is difficult to obtain good quality human embryos for research purposes and it is not permitted to use human embryos for research in some countries. Hence, most researchers use discarded human embryos from IVF clinics. Indeed, in IVF clinics, many poor quality human embryos have been discarded because they showed no survival characteristics at the end of culture.

Hardarson *et al.* (2003) found that 58% of the embryos produced by IVF had chromosomal abnormalities at blastocyst stage. These abnormal embryos can be used to derive hESC lines (Baharvand *et al.*, 2006). However, it is still unknown whether hESC lines with abnormal karyotypes have similar biological characteristics and functions to those with normal karyotypes. Therefore, in the present study, we used the discarded embryos to establish hESC lines and then compare the biological characteristics, imprinted gene expression, DNA methylation and X chromosome between the hESC lines with normal and abnormal karyotypes.

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Materials and Methods

Preparation of feeder layers

The feeder layers of murine embryonic fibroblasts (MEF) were prepared from Day 13.5 post-coitum fetuses of Kunming mice as previous described (Li *et al.*, 2004).

Culture of human embryos

This research was approved by the ethics committee of Guangzhou Medical College. Human embryos from IVF centers were donated on Day 3 after the patients signed the consent. The embryos were cultured in G2.3 medium (Vitrolife, Gothenburg, Sweden) until Day 5 (Kim *et al.*, 2005). On Day 5, early blastocysts were cultured for additional 2 days in a blastocyst optimum culture medium, which is G2.3 medium supplemented with 2000 U/ml of human recombinant leukemia inhibitory factor (hLIF; Chemicon, Temecula, CA, USA) and 10 ng/ml of human basic fibroblast growth factor (bFGF; Vitrolife).

Isolation of inner cell mass

Day 7 expanded blastocysts and hatched blastocysts were used to derive the ESC lines. Zona pellucida of expanded blastocyst was removed by treatment with 0.1% pronase (Sigma). The inner cell mass (ICM) of blastocysts were isolated by immunosurgery or mechanical method. Isolated ICMs were then placed on mitomycin C-treated MEF feeder layers for further culture.

Culture of hESCs

After the ICMs were seeded on the feeder layer, the formation of dome structure was examined after 8–9 days of culture. The ICMs were then mechanically broken down into 2–3 small clumps using a small pipette and the ICM clumps were transferred to a freshly prepared feeder layer. These cells were again mechanically dissociated during the initial five passages. After five passages, they were incubated in 1 mg/ml collagenase IV (Invitrogen) for 20–25 min at 37°C before further culture on freshly prepared feeders. The cells were routinely passed every 4–5 days, and the medium was changed every day. The hESC culture medium is knockout-Dulbecco's modified Eagle's medium (Gibco) supplemented with 15% serum replacement (GIBCO), 5% defined fetal bovine serum (Hyclone), 2 mM glutamine, 0.1 mM β-mercaptolethanol, 0.1 mM non-essential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, 4 ng/ml bFGF (Invitrogen) and 2000 U/ml hLIF. After 10 passages, hLIF was not added in the culture medium.

Karyotype analysis

For karyotype analysis, ESCs at passages 12, 22 and 32 were incubated in the culture medium with 0.25 μg/ml colcemid (Gibco) for 4 h, then with 0.4% sodium citrate and 0.4% chloratum Kaliumat (1:1, v/v) at 37°C for 5 min, and finally were fixed in methanol:acetic acid (3:1, v/v) solution. After Giemsa staining, at least 20 cells were examined in each group for the karyotype analysis.

Fluorescence in situ hybridization

For fluorescence *in situ* hybridization (FISH) analysis, ESC suspensions were dropped onto wet slides, dried at 63°C overnight and then dehydrated with ethanol in sequential concentrations of 70%, 85% and 100% before hybridization. FISH was performed using Vysis MultiVysion® PGT Multi-color Probe set (Vysis Inc., No. 32–131080), which includes five probes for chromosomes of 13, 18, 21, X and Y. The samples were stained according to recommended FISH protocols from manufacturer and examined under a fluorescence microscope. At least, 10 cells were examined in each cell line at each time of examination.

Staining for ESC markers

Human ESC marker staining was performed after 20 passages. To detect alkaline phosphatase (AP) activity, ESC colonies were fixed with 90% alcohol for 2 min, washed three times with Tween-BST solution [phosphate-buffered saline (PBS) with 1% bovine serum albumin and 0.2% Tween-20], and then stained with BCIP/NBT (AP substrate solution, Maxim Biotech Inc., USA.) for 30 min. To detect the hESC stage-specific embryonic markers, ESCs were fixed with 4% paraformaldehyde for 30 min and then incubated with 4% goat serum for 1 h before ESC marker staining. Primary antibodies were stage-specific embryonic antigens (SSEA)-4, SSEA-1, tumor-rejection antigen (TRA)-1-81 and TRA-1-60 (Chemicon). All antibodies were diluted 1:50 with PBS and the cells were incubated with antibody solution at room temperature for 1 h. The cells were washed three times with Tween-BST solution for 5 min and then incubated with the secondary antibody [goat anti-mouse immunoglobulin (Ig)G and goat anti-mouse IgM, both 1:100 dilution] conjugated to fluorescein isothiocyanate for 30 min. Negative controls were carried out without the addition of the primary antibodies. Hoechst 33342 was used for nuclear staining. The cells were then washed again and examined under a fluorescence microscope or confocal microscope.

Oct-4 expression

Total RNA was purified using Trizol Kit (Invitrogen) and RT–PCR reaction was carried out using Qiagen One Step for RT–PCR Kit (Qiagen, Germany) according to manufacturer's instructions. Octamer binding protein 4 (Oct-4) primers were used (Table I). RT–PCR was carried out by reverse transcription for 30 min at 50°C, initial PCR activation for 15 min at 95°C, followed by 30 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 54°C and finally extension for 1 min at 72°C. The PCR amplified

Table I. RT–PCR and methylation-specific PCR primer sequences.

Gene	Primer forward 5′-3′	Primer reverse5′-3′	Size (bp)
Oct-4	GTGTTCAGCCAAAAGACCATC	CCCTGAGAAAGGAGACCCA	387
H19	CCGGACACAAAACCCTCTAGCT	TGTTCCGATGGTGTCTTTGATG	142
IGF2	TCCCCTGATTGCTCTACCCA	GCAGTTTTGCTCACTTCCGATT	86
SNRPN	TGGCACCTTTAAGGCTTTTG	CCG CTTTTCTTCACGCTCT	112
GNAS	CAGCACTGCCAGTGGAGATG	TGTCACGGCAGTCGTTGAAC	101
GAPD	GGAGTCAACGGATTTGGTCG	CCTGGAAGATGGTGATGGG	218
SNRPN-M	TAAATAAGTACGTTTGCGCGGTC	AACCTTACCCGCTCCATCGCG	177
SNRPN-P	GTAGGTTGGTGTGTATGTTTAGGT	ACATCAAACATC TCC AACAACCA	100

Oct-4, octamer binding protein 4; IGF2: insulin-like growth factor; SNRPN, small nuclear ribonucleoprotein polypeptide N; GAPD, glyceraldehyde-3-phosphate dehydrogenase; SNRPN-M, used to analyze methylated status; SNRPN-P, used to analyze unmethylated sites.

products were analyzed on 1.5% agarose gel and visualized by ethidium bromide (Invitrogen) staining.

DNA fingerprinting and HLA typing

Total DNA was extracted using Qiagen DNeasy Tissue Kit (Qiagen) according to manufacturer's instructions. Extracted DNA was amplified for 16 different genetic loci using the Promega PowerPlex 16 System kit (Promega, USA). Capillary electrophoresis was carried out on an automated ABI 3100 Genetic Analyzer (Applied Biosystems). The 16 short tandem repeat (STR) loci were D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, amelogenin, vWA, D8S1179, TPOX and FGA.

HLA typing was performed by PCR with sequence specific primers (Biotest, Landsteinerstr, Dreieich Germany, Biotest HLA SSP Kit http://www.biotest.de). The products were identified using agarose gel electrophoresis followed by the detection of the DNA bands in UV light with the aid of the Biotest SSP typing software to determine the HLA-A, HLA-B and HLA-DR loci. All manipulations were performed according to manufacturer's recommendations.

Differentiation assessment in vitro

The ESC colonies were dissociated with 1 mg/ml collagenase IV and cultured in culture plates to prevent attachment of the cells. After culture for 3 days, the cells were transferred to a new culture plate. Seven days after culture, the formation of embryoid bodies (EBs) was examined. EBs were transferred to 0.1% gelatin-coated culture dish for spontaneous differentiation. The differentiated cells were stained with antibodies against human smooth muscle actin, cardiac troponin I, alpha fetoprotein and nestin (Chemicon). The EB culture medium was the same as hESC culture medium but without bFGF and hLIF.

Differentiation assessment in vivo

The ESC colonies of passage 15 or beyond were harvested and were broken down into 300–400 small ESC colony suspension. The colonies were injected into inguinal groove of 6-week-old male severe combined immunodeficiency (SCID) mice. Twelve weeks later, the resultant tumors were removed, fixed in 4% paraformaldehyde and embedded in paraffin. Sections were prepared, stained with hematoxylin and eosin, and examined for the presence of tissues derived from the three germ layers.

Analysis of imprinted genes in undifferentiated hESCs

In order to identify the imprinted gene expression in undifferentiated hES cells, total RNA was extracted from different hESC lines. Gene expression pattern in undifferentiated cells was profiled using the QIAGENE one step RT–PCR kit. Selected imprinted genes were H19, insulin-like growth factor (IGF)2, small nuclear ribonucleoprotein polypeptide N (SNRPN) and the conditional gene GNAS (Table I). The PCR was performed using 50°C for 30 min, 95°C for 15 min and followed by 94°C for 30 s, 55°C for 30 s and 72°C for 45 s for 45 cycles and 72°C for 5 min (Sun *et al.*, 2006). The PCR products were analyzed by 2% polyacrylamide gel electrophoresis, stained with ethidium bromide and documented using the BioImaging system (UVP, Upland, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase served as a ubiquitously expressed control. Genomic contamination was ruled out by including an RT-negative sample in each PCR set as a control.

DNA methylation analysis

Methylation patterns of the imprint control (IC) region of the human SNRPN-gene (Table I) were studied in the undifferentiated hESCs. Four hESC lines, FY-hESC-1 (46, XY), -5 (unbalanced Robertsonian translocations), -8 (46, XX) and FY-3PN (69, XXX), were analyzed. Prader–Willi syndrome (PWS) and Angelman syndrome (AS) patients as well as normal DNA samples were also analyzed by using methylation-specific PCR (MSP) assay (Kubota *et al.*, 1997). Genomic DNA was extracted according to the manufacturer's instructions (QIAamp DNA Blood Mini Kit). The PCR products were analyzed by 7% polyacrylamide gel electrophoresis.

X chromosome inactivation status

Human androgen receptor gene contains a highly polymorphic trinucleotide repeat in the first exon. It has been found that the methylation of HpaII and HhaI sites <100 bp away from this polymorphic STR correlates with X inactivation. MSP was used to determine the methylation status of the selected sample with XX chromosome (FY-hES-5, -7, -8 and FY-3PN). Genomic DNA was extracted from the XX hESC lines. Two sets of PCR were prepared. One was for methylated X alleles and the other was for unmethylated alleles. MSP primers were Primer ARM-F 5′-GCG AGC GTA GTA TTT TTC GGC-3′, Primer ARM-R 5′-AAC CAA ATA ACC TAT AAA ACC TCT ACG-3′, Primer ARU-F 5′-GTT GTG AGT GTA GTA TTT TTT GGT-3′ and Primer ARU-R 5′-CAA ATA ACC TAT AAA ACC TCT ACA-3′. Amplification and gel analysis were performed as manufacturer's instruction. Bisulfite-converted CpGenome Universal Methylated DNA (Chemicon http://www.chemicon.com) and bisulfite-converted female human blood DNA were used as positive controls. Sample tubes were loaded to Genetic Analyzer 310 for analysis of fragmentation. The size of PCR products of the androgen receptor gene was between 177 and 221 bp (Kubota *et al.*, 1999).

Results

Derivation of hESC lines

In this study, 265 donated embryos were used and 42 (15.8%) developed to early blastocysts on Day 5 (Fig. 1I, A). When these early blastocysts were transferred to blastocyst optimum culture medium for another 2 days, 36 developed to expanded blastocysts and 6 to hatched blastocysts. A total of 42 ICMs (Fig. 1I, B) were isolated using immunosurgery (19 ICM) or mechanical method (23 ICM). All ICMs were seeded on MEF feeder layer (Fig. 1I, C-H).

Seven hESC lines have been established in our laboratory (16.7% of the blastocysts or 2.6% of Day 3 embryos). After nine passages, cells at various passages were frozen and thawed to examine the survival status and were found to survive in the subsequent cultures. FY-hES-1 has been in continuous culture for 1 year and 76 passages, whereas FY-3PN, FY-hES-3, -4, -5, -7 and -8 have been in continuous cultures for 44, 38, 30, 27, 20 and 15 passages, respectively (Table II).

Characterization and identification of hESC lines

Cells in all seven lines showed a high level of AP activity and strongly expressed TRA-1-60, TRA-1-81 and SSEA-4 (Fig. 1II, A, D, E and C, respectively) but not SSEA-1 proteins (Fig. 1II, B). Oct-4 mRNA expression was observed in all seven hESC lines (Fig. 2). Sixteen STR loci were analyzed for hESC lines and each cell line showed distinct STR loci indicating that they were derived from different embryos (Fig. 3A–G). HLA typing also showed that the seven lines have different HLA-A and DBR loci (Table III).

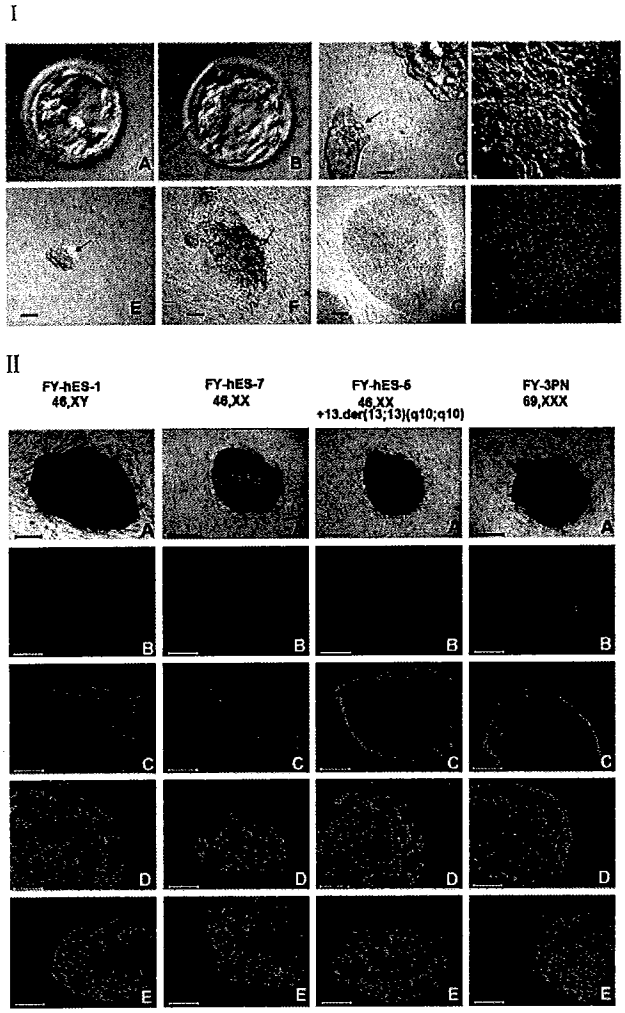

Figure 1: Derivation of hESC lines and characteristics. (**I**) Human embryos and isolated ICM. (A) A Day 5 blastocyst cultured in G2.3 blastocyst medium. Note the small ICM. (B) A Day 7 blastocyst that has been cultured in the blastocyst optimum medium for another 2 days. The ICM became clearer. (C) An ICM isolated by mechanical method. (D) A round ICM colony surrounded by a group of residual trophectoderm after mechanical isolation. (E) An ICM isolated by immunosurgery. (F) A dome-like structure on the feeder layer formed after 8 days culture of ICM isolated with immunosurgery. Arrows in C to F indicate ICMs. (G) Typical round hESC colonies with very clear boundary at low magnification. (H) hESC morphology in a colony, showing a high nucleus–cytoplasm ratio; note the presence of nucleoli and typical intercellular spaces at high magnification. Bar = 25 μm in A, B, C and D and bar = 100 μm in E, F and G. (**II**) Immunocytochemical staining of undifferentiated hESC colonies after 32 passages in four cell lines (A) AP, (B) SSEA-1, (C) SSEA-4, (D) TRA-1-60 and (E) TRA-1-81. Bar = 100 μm.

Karyotypes of the hESC lines

Chromosome analysis and FISH examination showed that FY-hES-1, -3 and -4 had normal 46, XY karyotypes, FY-hES-7 and -8 had normal 46, XX karyotypes, FY-3PN had 69, XXX karyotype (Fig. 4A–F), whereas FY-hES-5 was an unbalanced Robertsonian translocations with 46, XX,+13, der(13;13)(q10;q10) (Fig. 4G) irrespective of the passages 12, 22 and 32. As shown in Fig. 4H, FISH images of FY-hES-5 at the 22 passage showed three chromosome 13 (red signals), 2 chromosome 18 (aqua), 2 chromosome 21 (green), 2 X chromosome (blue) and no Y chromosome after five probe staining, which was the same as examined in other passages. All cells in other cell lines also maintained the same karyotypes as original chromosomal analysis. Embryo donor for FY-hES-5 had normal karyotype.

Differentiation of hESC lines

The cells of the hESC lines were cultured in suspension on Petri dishes, simple EBs were formed on Day 3 and cystic EBs on Dasy 6–7. On Day 8, these EBs were transferred to 0.1% gelatin-coated plates for further culture to examine cell differentiation. After 4 days culture, these cells were positively stained by antibodies against human smooth muscle actin (mesoderm), alpha fetoprotein (endoderm) and nestin (ectoderm) (data not shown). Hence, all hESC lines were able to differentiate into three germ layers *in vitro*. When hESCs were injected into SCID mice, teratomas were first observed at 4–5 weeks and the size of teratomas reached 25 × 30 mm after 12 weeks. After the teratomas were excised and sectioned for examination, three embryonic germ layers including endoderm (gut epithelium), mesoderm (cartilage and muscle) and ectoderm (squamous epithelium, neuroectoderm and neural ganglia) were identified (Fig. 5A–D).

Analysis of imprinted genes in undifferentiated hES cells

In order to assess if there are any differences in gene expression between normal and abnormal karyotype hESC lines, we examined maternal expressed imprinted geneH19, paternal expressed imprinted gene IGF2 and SNRPN and conditional gene GNAS as disrupted expression of these genes is associated with human genetic diseases, such as PWS and AS. We found that gene expression patterns in all four of these cell lines were similar. These results indicate that expression of H19, IGF2, SNRPN and GNAS in the abnormal karyotype hESC lines were regulated in a similar way as in the normal ESC lines (Fig. 6).

DNA methylation

The SNRPN is a paternally expressed imprinted gene that is located on chromosome 15q11–13, a region related to PWS and AS. The IC-region of the SNRPN-gene showed 23 CpG-sites that are methylated on the maternal chromosome and unmethylated on the paternal chromosome (Zeschnigk *et al.*, 1997). Genomic sequencing of the SNRPN region after bisulfite treatment has revealed that >96% of all CpG dinucleotides are methylated on the maternal chromosome, but none on the paternal chromosome. A normal person or normal hESCs have both methylated and unmethylated SNRPN gene sites. In this study, two pairs of primers were used to analyze SNRPN gene methylation status. Primer SNRPN-M was used to analyze methylated status (174 bp band) and primer SNRPN-P was used to analyze unmethylated sites (100 bp band). Normal and abnormal karyotype hESC lines showed both 100 and 174 bp bands. MSP analysis

Table II. Human embryonic stem cell (hESC) lines and their characteristics.

hESC lines	FY-hES-1	FY-hES-3	FY-hES-4	FY-hES-5	FY-hES-7	FY-hES-8	FY-3PN
Karyotypes	46, XY	46, XY	46, XY	46, XX, +13,der (13;13) (q10;q10)	46, XX	46, XX	69,XXX
AP activity	+	+	+	+	+	+	+
SSEA-4	+	+	+	+	+	+	+
SSEA-1	−	−	−	−	−	−	−
TRA-1-81	+	+	+	+	+	+	+
TRA-1-60	+	+	+	+	+	+	+
STR	+	+	+	+	+	+	+
HLA typing	+	+	+	+	+	+	+
FISH analysis	+	+	+	+	+	+	+
Embryoid *in vitro*	+	+	+	+	+	+	+
Teratomas *in vivo*	+	+	+	+	+	+	+
Imprinted genes	√	√	√	√	√	√	√
DNA methylation	√	N/A	N/A	√	√	N/A	√
X chromosome status	√	N/A	N/A	√	√	√	√
No. of Passages	76	38	30	27	20	15	44

AP, alkaline phosphatase; SSEA, stage-specific embryonic antigen; TRA, tumor-rejection antigen; STR, short tandem repeat; FISH, fluorescence in-situ hybridization; "√" indicates that the samples were analyzed; N/A, not applicable (not analyzed).

demonstrated that all of the hESC lines have a normal SNRPN methylation status (Fig. 6).

Determination of X-inactivation pattern

X-inactivation means that one of the X chromosomes is silenced in XX female mammals. Initiation of this process during early development is controlled by the X-inactivation centre, a complex locus that determines how many and which X chromosomes will be inactivated. In order to analyze DNA methylation in XX hESC lines, MSP was used to observe the X-inactivation status in these ESC lines (Table II).

In a random X chromosome inactivation pattern, the XX hESC should have two active alleles and two inactive alleles. The peak area ratio in both active and inactive allele should be 50:50 (the peak area ratio of the small allele to the larger allele). However, in the present study, we found that all of the XX hESC lines have both active and inactive X chromosomes with non-random inactivation patterns of either >80:20 or <20:80 (Fig. 7). FY-hES-5 and -8 had almost complete non-random X chromosome inactivation patterns with only one inactive X chromosome (194 bp in FY-hES-5 and 191 bp in FY-hES-8). The inactivation ratio in the FY-3PN was also non-random (data not shown). In contrast, in the normal female blood DNA samples (as a control), there were two active (194 and 207 bp) and two inactive (194 and 207 bp) X chromosomes with a random X chromosome inactivation pattern of 50:50. The XY hESC line (FY-hES-1) always had one active X chromosome (197 bp) but did not have inactive X chromosome (Fig. 7).

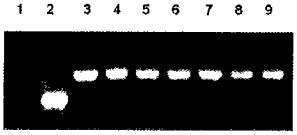

Figure 2: Octamer binding protein 4 expression by RT–PCR. Lanes 3–9 represent FY-hES-1, -3, -4, -5, -7, -8 and FY-3PN, respectively. Lane 1 is a negative control and lane 2 is human β-actin (265 bp).

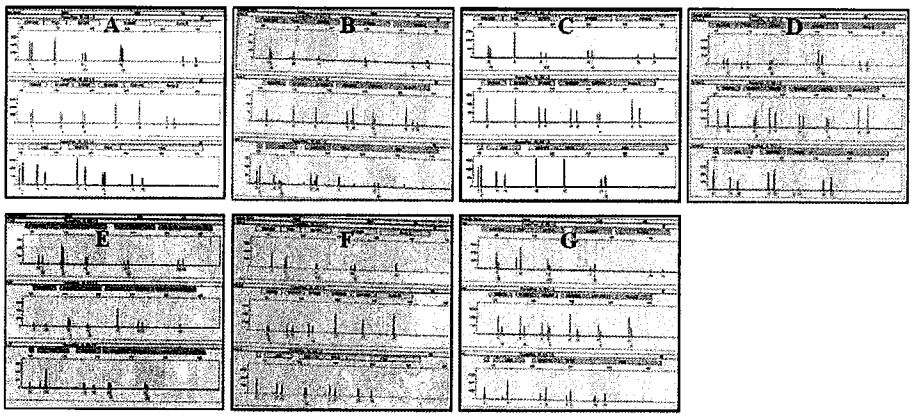

Figure 3: DNA fingerprinting of FY-hES-1 (**A**), -3 (**B**), -4 (**C**), -5 (**D**), -7 (**E**), -8 (**F**) and -3PN (**G**), respectively.

Table III. HLA typing of FY-hESC lines.

HLA type	A	B	DRB
FY-hES-1	A2/A2	B46/B60	DR9/DR16
FY-hES-3	A11/A11	B46/B46	DR9/DR11
FY-hES-4	A2/A2	B46/B54	DR9/DR10
FY-hES-5	A11/A29	B7/B54	DR10/DR13
FY-hES-7	A11/A24	B35/B35	DR14/DR15
FY-hES-8	A11/A33	B13/B58	DR9/DR16
FY-3PN	A11/A33	B44/B58	DR17/DR10

Discussion

In the present study, five hESC lines with normal karyotypes and two lines with abnormal karyotypes have been derived from poor quality blastocysts. Successful derivation of hESC lines from poor quality blastocysts has been previously reported by other authors (Hovatta *et al.*, 2003; Mitalipova *et al.*, 2003; Genbacew *et al.*, 2005; Chen *et al.*, 2005; Mateizel *et al.*, 2006; Lerou *et al.*, 2008): the derivation rate from poor quality blastocysts was <10%. In the present study, out of 42 blastocysts, seven ESC lines have been established with a rate of 16.7%. This higher rate may be attributed to the further culture of Day 5 blastocysts in the blastocyst optimum culture medium, which significantly increased the number of cells in the ICM, thus isolation of ICM became much easier. However, if the rate is calculated from Day 3 embryos, it is very low (2.6%) in the present study. This rate is the same as that reported previously (Chen *et al.*, 2005; Genbacew *et al.*, 2005). The low rate is due to poor quality of Day 3 embryos as many of them usually arrest during the subsequent culture due to chromosome abnormalities, such as aneuploidy, mosaicism, haploidy or polyploidy, as these are often found in poor human embryos (Magli *et al.*, 2007; Munne *et al.*, 2007).

Currently, there are standard culture protocols for human ESC culture and there are many poor quality human embryos being discarded from IVF clinics. Thus, if these embryos can be used correctly, it is possible to establish more and more hESC lines.

We also established two ESC lines with abnormal chromosomal constitution (FY-hESC-5) and three pronucleus (FY-3PN) embryos. Some hESC lines with abnormal chromosomal constitution have also been derived previously (Draper *et al.*, 2004; Heins *et al.*, 2004; Munne *et al.*, 2005; Verlinsky *et al.*, 2005; Baharvand *et al.*, 2006). Munne *et al.* (2005) reported that ESCs derived from trisomic embryos can undergo self-correction, partially or totally, to chromosomally normal cells, thus they observed mosaic in their hESCs. However, we did not observe such self-correction in the present study.

There are two possibilities for the origins of cell lines with normal karyotype. One is that it is derived from normal fertilized oocytes, thus all chromosomes are normal in the subsequent culture, and the other is that it is derived from embryos with chromosomal abnormalities, but the cells

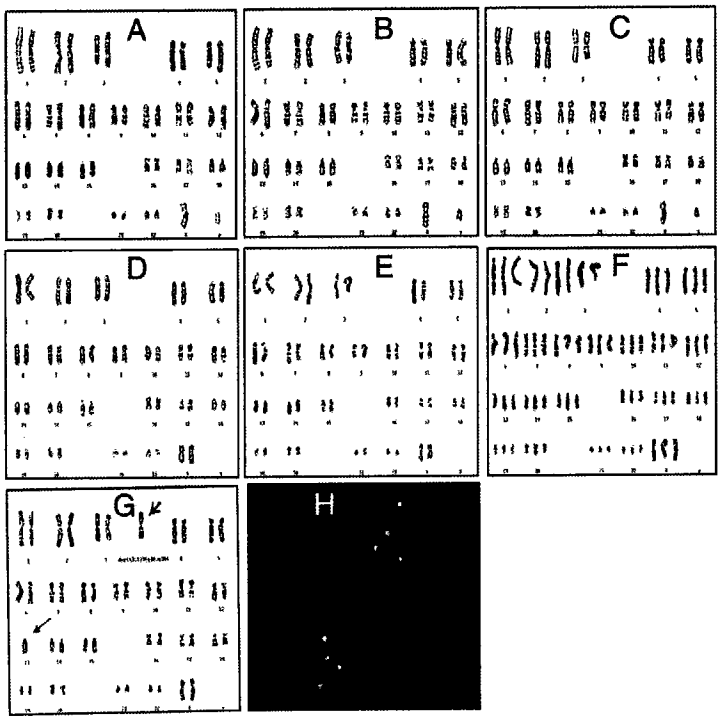

Figure 4: Karyotypes of hESC lines.
Karyotype analysis (**A**–**G**) and FISH images (**H**) of hESC. Normal 46, XY karyotypes of FY-hES-1 (A), -3 (B) and -4 (C), normal 46, XX of FY-hES-7 (D) and -8 (**E**). FY-3PN (**F**) shows a triploid karyotype of 69, XXX. FY-hES-5 (**G**) shows unbalanced Robertsonian translocations with 46, XX+13, der (13;13) (q10;q10) karyotype. Blue arrow indicates duplicate chromosome 13. FISH of interphase nuclei from FY-hES-5 cells shows two chromosome 13 (red), two chromosome 21 (green), two chromosome 18 (aqua) and two X chromosomes (blue) (H).

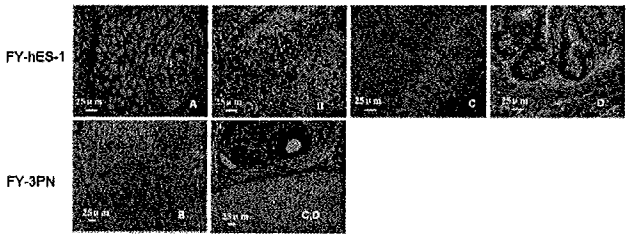

Figure 5: Histology analysis of teratomas derived from FY-hES-1 and FY-3PN hESC lines.
(**A**) Squamous epithelium tissue from ectoderm; (**B**) neural ganglia tissue from ectoderm; (**C**) cartilage tissue from mesoderm and (**D**) glandular tissue from endoderm.

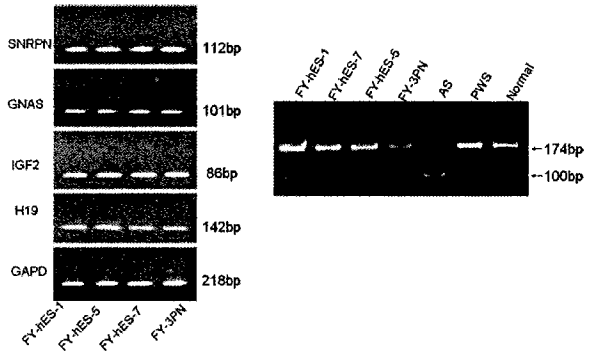

Figure 6: RT–PCR analysis of imprinted gene expression (left panel).
Paternally expressed gene IGF2, small nuclear ribonucleoprotein polypeptide N (SNRPN) and maternally expressed gene H19 were analyzed in four hESC lines with different karyotypes. The conditional gene GNAS was used as an additional control. Glyceraldehyde-3-phosphate dehydrogenase (GAPD) was included as mRNA quantitative control. SNRPN gene methylation status (right panel) in FY-hES-1, -5, -7, and -3PN lines, AS, PWS and normal DNA was used as control.

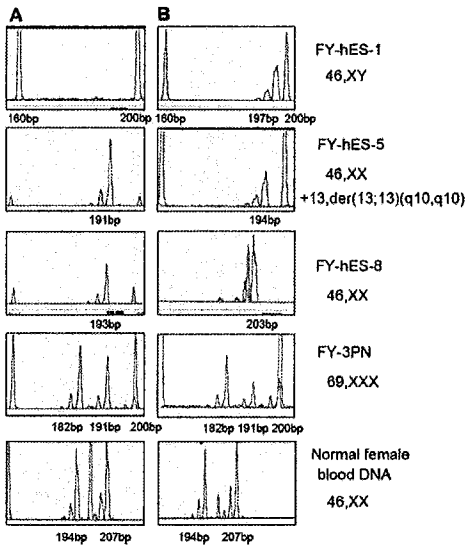

Figure 7: X chromosome inactivation analysis of the XX hESC line. Column A represents inactive X chromosome and column B represents active X chromosome. A normal female blood DNA and FY-hES-1 (46, XY) was used as a control (red peak is the marker and blue peak is the product).

undergo a self-correction during subsequent culture, thus the chromosomes are normal in the cell lines. In the present study, because we did not examine the embryo's chromosomal constitutions at Day 3 or 5, we do not know if the cell lines with normal karyotype are derived from embryos with normal chromosomes or embryos with chromosomal abnormalities.

In the present study, self-correction was not observed in a hESC line (FY-3PN) that was derived from a triploid embryo. Triploid embryos used for hESC line derivation may be from a polyspermic oocyte or a diploid oocyte plus a fertilized sperm. In the present study, when we further examined STRs in this cell line, we found that it was a homogeneous triploid cell line (the peak area ratio of each STR locus except D7S820 is almost 1:2.) (Fig. 3G). Therefore, this cell line may result from duplication of the chromosomes in the oocyte. No mosaic in the chromosomal constitutions may indicate that the chromosomes are more stable in the cell lines derived from such triploid embryos than trisomy embryos.

Also, in the unbalanced Robertsonian translocation cell line, the two long arms of 13 chromosomes fused at the centromere and the two short arms were lost. This situation is also different from chromosome separation error in the aneuploid embryo. Thus, it may be difficult to self-correct such an error. From these results, it would appear that self-correction occurs in the ESCs derived from partial chromosomally abnormal embryos, but not in the ESC lines derived from complete triploid embryos or translocation embryos. It is also possible that some cell lines may undergo self-correction, but others may not.

Previous studies indicated that hESC from chromosomally abnormal embryos had all cell markers that hESC should have, and had the ability to differentiate (Heins *et al.*, 2004; Baharvand *et al.*, 2006). In the present study, we examined not only cell markers and ability to differentiate, but also other characteristics, such as imprinted genes, DNA methylation and X chromosome inactivation. Similar to previous studies, we did not find any difference in all characteristics in all seven lines, such as AP activity and cell surface markers, SSEA-4, TRA-1-60 and TRA-1-81. Oct-4, which is an important factor for early embryos and undifferentiated cells (Hay *et al.*, 2004: Lee *et al.*, 2006), was present in all cell lines.

Furthermore, all of these hESC lines are pluripotent. When the cells were injected into immunosuppressed mice to examine the formation of teratomas, we found that they formed cells of all three germ layers including mesoderm (cartilage), ectoderm (epithelium) and endoderm (muscle cells). Also when these cells were spontaneously differentiated *in vitro*, EBs formed muscular cells, nerve cells and other types of cells.

In order to practically use the established hESCs, the characteristics of each cell line should be clarified. Therefore, we developed a comprehensive database of DNA profiles for each cell line based on STR loci and HLA typing. The exploitation of STR elements in the genome is important in the field of genetic mapping, linkage analysis and human identity testing. STR loci have become the standard for identifying hESC lines (Plaia *et al.*, 2006). STR analysis is also useful in confirming and clarifying some of the anomalies. For

example, STR map of FY-3PN showed different peak mapping when compared with others. In order to provide more major histocompatibility complex matched cell lines, HLA typing would be critical for stem cell-based therapies. HLA is a family of cell proteins found on the surface of white blood cells and other nucleated cells in the body. These proteins vary from person to person and are critical for the activation of immune responses. HLA-matched transplantation will minimize the possibility of rejection. Our results from seven cell lines revealed that all of these cells were heterozygous HLA genotype.

Epigenetic stability has profound implications for the use of hESCs in regenerative medicine. Genomic imprinting is erased in the primordial germ cells during development and is reestablished during gametogenesis. Aberrant expression of imprinted genes can cause inherited diseases and induce tumors. For example, the imprinting domain on human chromosome 15q11–13 contains a large cluster of imprinted genes, including paternally expressed SNRPN. Improper regulation of imprinted genes in this cluster results in PWS and AS (Glenn *et al.*, 1997). Loss of imprinting of H19 gene or IGF2 gene, which is normally located at 11p15.5, is related to embryonic cancers, such as BWS [Beckwith–Wiedemann syndrome and Wilm's tumor, Neu-roblastomas and Yolk sac carcinomas (Rainier *et al.*, 1995)]. In our study, no different expression in H19, IGF2, SNRPN and GNAS was found in these cell lines.

DNA methylation is essential for normal mammalian development (Herman *et al.*, 1996). It is not clear whether the potential epigenetic changes occur during long-term ESC culture. Thus, in order to reveal epigenetic stability of imprinted genetic regions of SNRPN in undifferentiated hES cells, we examined DNA methylation status via MSP (Zeschnigk *et al.*, 1997). The SNRPN critical region, such as the maternal allele, is methylated and the paternal allele is not methylated and is transcriptionally active. MSP analysis of these regions demonstrated that all of the hESCs have a normal SNRPN methylation status, indicating that there are no deletions, uniparental disomy or imprinting mutations of SNRPN methylation in these normal and abnormal karyotype hESC lines.

In mice, establishing a stable XX ESC line is not easy due to loss of one X chromosome. The unstable X chrosomosome and DNA methylation have been found in diploid parthenogenetic ESC lines, which results in only one X chromosome (XO genotype) in the cells (Robertson *et al.*, 1983; Zvetkova *et al.*, 2005). Failures of X chromosome inactivation in different hESC lines has also been reported (Dhara and Benvenisty, 2004; Hoffman *et al.*, 2005). Epigenetic variation between hESC lines may also perturb X chromosome inactivation. In order that female embryos express similar levels of X-linked genes to males, one of the two X chromosomes is inactivated at an early embryonic stage. It has been revealed by genetic studies that X chromosome selection is influenced by the X controlling element (Simmler *et al.*, 1993). The X chromosome is randomly inactivated in a 50:50 ratio in most females whereas ~10% females have a non-random X chromosome inactivation (Kubota *et al.*, 1999). Asymptomatic female carriers of X-linked diseases have the preferential selection of the normal non-mutated X chromosome, which causes extremely non-random inactivation, such as X-linked hyper-IgM syndrome (97:3), Pai syndrome (89:11), multiple congential anomalies (4:96) and unbalanced X autosome translocation (91:9) (Kubota *et al.*, 1999). Therefore, determination of the X-inactivation pattern is important for the detection of carriers of X-linked diseases. Our data showed that all of our XX hESC lines have both active and inactive X chromosomes. Almost extremely non-random X chromosome inactivation patterns (>95:5) were also found in FY-hES-5, FY-hES-8 and FY-3PN. Our data indicate that the patterns of XX hESC with extremely non-random X chromosome inactivation are similar to the patterns of X-linked diseases with skewed X chromosome inactivation. Whether this phenotype means a relationship between XX hESC lines and X-linked disease is unknown, thus further study on the mechanism of non-random X inactivation may be necessary to explain epigenetic states and developmental competence in hESC lines. X chromosome inactivation should be affected in the hESC lines derived from triploidy embryos since there were three X chromosomes. However, we did not find significant differences in X chromosome inactivation in the cell line with XX chromosomes and three X chromosomes. Further studies are necessary to address these issues and hESC lines with abnormal karyotypes are useful materials for these studies.

In conclusion, our results indicate that new hESC lines can be successfully established from poor quality human embryos. All hESC lines established in our laboratory showed all hESC characteristics and could be differentiated into three germ layers, regardless of their karyotype. Through the detailed examination of ESC biological characterizations, gene expression, DNA methylation and X-inactivation, we found that hESC lines with abnormal karyotype are also useful experimental materials for developmental biology and genetic research. Our results also indicate that these ESC lines have potential application in human cell therapy.

Acknowledgements

We gratefully acknowledge the support of Dr Y.W. Kan from University of California for critically reading of the manuscript.

Funding

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Submitted on January 11, 2008; resubmitted on March 16, 2008; accepted on April 1, 2008

Markers used to characterise cell line and result *(Indicate passage level at which marker studies were carried out)*

FY-hES	1	3	5	7	8	3PN
Passage	76	38	27	20	15	44
AP Activity	+	+	+	+	+	+
OCT4	+	+	+	+	+	+
SSEA-1	-	-	-	-	-	-
SSEA-4	+	+	+	+	+	+
TRA-1-60	+	+	+	+	+	+
TRA-1-81	+	+	+	+	+	+
HLA	+	+	+	+	+	+
Embryoid body in vitro	+	+	+	+	+	+
Karyotype	46,XY	46,XY	46,XX +13,der,(13;13)(q10;q10)	46,XX	46,XX	69XXX
STR	+	+	+	+	+	+
Teratoma	+	+	+	+	+	+

These cell lines were published in Human Reproduction :

Sun X, Long X, Yin Y, Jiang Y, Chen X, Liu W, Zhang W, Du H, Li S, Zheng Y, Kong S, Pang Q, Shi Y, Huang Y, Huang S, Liao B, Xiao G, Wang W. Similar biological characteristics of human embryonic stem cell lines with normal and abnormal karyotypes. Hum Reprod. 2008 ,23(10):2185-93.

(29)

From: 筱放
To: HESCREGISTRY (NIH/NIDCD)
Subject: RE: New hESC Registry Application Request #2010-ACD-002
Date: Wednesday, June 30, 2010 9:12:22 PM

Dear Dr.Ellen Gadbois,

1. Please clarify the timing of embryo donation consent process. Were all embryos donated on Day 3 post-fertilization? If not, for each donor couple, when did clinical IVF treatment occur relative to embryo donation?

: Yes , all the embryos donated on Day 3 post-fertilization. Embryos were morphologically graded, as described by Edwards as follows grade 1, equal-sized symmetrical blastomeres; grade 2, uneven blastomeres with < 10% fragmentation; grade 3, (10-50%) blastomeric fragmentation; and grade 4, (>50%) blastomeric fragmentation embryos. Only grade 3 and grade 4 embryos that were not used for transfer or freezing on day3 were uesd after obtaining the cumulative embryo score (CES). *

Were all of these donated embryos poor-quality embryos (i.e. not suitable for IVF clinical use)?

Answer : Yes, all the donated embryos are poor-quality embryos. *

Please clarify whether use of the human embryonic stem cell lines by another individual or research institution requires reconsent from the donors of the embryos. (Note: the English translation provided by the Guangzhou Medical College contains the statement " The discarded embryos can not be available to any individual or research units without our consent." NIH translators independently translated the same sentence as "The specimens may not be provided to any individual or research institution without our consent.")
Answer : In our consent, we just use the the discarded embryos to produce human embryonic stem cell lines. After we established the human embryonic stem cell lines from the discarded embryos, the human embryonic stem cells can be available to the researchers all over the world. But,we can not give the embryos to any individual or research units without the patients consent.

Was the same IVF clinical treatment consent form signed by all of the couples who donated embryos for research?
Answer : Yes, the same IVF clinical treatment consent form signed by all of the couples who donated embryos for research. *

Thank You!

Sincerely,
XiaoFang Sun

From: hescregistry@mail.nih.gov
To: xiaofangsun@hotmail.com; stemcellresearch@yahoo.cn
CC: hescregistry@mail.nih.gov
Date: Tue, 29 Jun 2010 13:38:23 -0400
Subject: RE: New hESC Registry Application Request #2010-ACD-002

Hello Dr. Sun,

The NIH Working Group for Human Embryonic Stem Cell Eligibility Review has begun its consideration of this submission under Section IIB of the NIH Guidelines for Human Stem Cell Research. The Working Group has asked if you can answer the following questions:

1. Please clarify the timing of embryo donation consent process. Were all embryos donated on Day 3 post-fertilization? If not, for each donor couple, when did clinical IVF treatment occur relative to embryo donation?
2. Were all of these donated embryos poor-quality embryos (i.e. not suitable for IVF clinical use)?
3. Please clarify whether use of the human embryonic stem cell lines by another individual or research institution requires reconsent from the donors of the embryos. (Note: the English translation provided by the Guangzhou Medical College contains the statement " The discarded embryos can not be available to any individual or research units without our consent." NIH translators independently translated the same sentence as "The specimens may not be provided to any individual or research institution without our consent.")
4. Was the same IVF clinical treatment consent form signed by all of the couples who donated embryos for research?

Please let us know if you have any questions about this request.

Sincerely,

(30)

Ellen Gadbois

Ellen L. Gadbois, Ph.D.
Office of Science Policy Analysis
Bldg 1 Room 218D
National Institutes of Health
voice: 301-594-2567
fax: 301-402-0280

From: HESCREGISTRY (NIH/NIDCD)
Sent: Thursday, February 25, 2010 9:30 PM
To: xiaofangsun@hotmail.com; xiaofangsun@hotmail.com
Subject: New hESC Registry Application Request #2010-ACD-002

To: THE THIRD AFFILIATED HOSPITAL OF GUANGZHOU MEDICAL COLLEGE (Signing Official)
xiaofangsun (Submitter)
This is to confirm that the hESC Registry Application request, as detailed below, has just been submitted and is pending review by the Working Group of the Advisory Committee to the Director (ACD). You can expect to hear back from us about the status of your application soon.

While pending review, the name of the stem cell line (Question 6) will appear on the public NIH Human Embryonic Stem Cell Registry for Submitted hESC Lines Pending Review.

After review, your organization name, name of the stem cell line(s) and the method of NIH review (Administrative Review or review by the Working Group of the Advisory Committee to the Director) will be posted on the Web page of hESC Lines Reviewed for NIH Funding Eligibility.

If the cell line is approved for inclusion on the NIH Registry, information entered into Questions 6-9 will be posted on the NIH Human Embryonic Stem Cell Registry for hESC Lines Eligible for NIH Funding.

If you have any questions about the hESC Registry Application process, please contact us.

Thank you,
hESC Registry Help Desk
hescregistry@mail.nih.gov

hESC Registry Request Information
for Advisory Committee to the Director (ACD) Review

Request #:	**2010-ACD-002** (Previously: 2010-DRAFT-006)
Status:	**Pending** (02/25/2010)
Administrative Information:	
1. Signing Official (SO):	**A.** Name: THE THIRD AFFILIATED HOSPITAL OF GUANGZHOU MEDICAL COLLEGE **B.** Phone: 862081292013 **C.** Email: xiaofangsun@hotmail.com
2. Submitter of Request:	**A.** Name: xiaofangsun **B.** Phone: 862081292013 **C.** Email: xiaofangsun@hotmail.com
3. Organization Name and DUNS	THE THIRD AFFILIATED HOSPITAL OF GUANGZHOU MEDICAL COLLEGE DUNS: 527189518

(31)

From: 孙筱放
To: HESCREGISTRY (NIH/NIDCD)
Subject: RE: New hESC Registry Application Request #2010-ACD-002
Date: Thursday, July 08, 2010 4:06:18 AM

Dear Dr.Ellen Gadbois,

When were the couples provided information about the stem cell research protocol?
Answer: When the couples sign the embryo donation consent form, the stem cell research protocol were provided. *

When did the IVF treatments occur?
Answer: Each patient underwent a basic physical examination before ovulation induction, including tests for human immunodeciency virus, hepatitis B virus, hepatitis C virus and contagious venereal disease. After all the examination finished, the IVF treatments occurred. *

When did the couples actually sign the actual embryo donation form, relative to their IVF treatments?
Answer: After all the examination finished, the couples sign their IVF treatments consent form and embryo donation consent form. *

Thank You!

Sincerely,
XiaoFang Sun

From: hescregistry@mail.nih.gov
To: xiaofangsun@hotmail.com
CC: hescregistry@mail.nih.gov
Date: Wed, 7 Jul 2010 09:44:13 -0400
Subject: RE: New hESC Registry Application Request #2010-ACD-002

Dear Dr. Xiao Fang Sun,

Thank you for your responses and the additional information.

The Working Group has one additional question to help them understand better when the embryo donors made certain decisions. In particular, the Working Group would like to understand when the donors signed the consent to donate embryos compared to when their IVF treatments occurred.

For each couple donating embryos, can you please explain:
When were the couples provided information about the stem cell research protocol?
When did the IVF treatments occur?
When did the couples actually sign the actual embryo donation form, relative to their IVF treatments?

Please let us know if you have any questions about this request.

Sincerely,
Ellen Gadbois

Ellen L. Gadbois, Ph.D.
Office of Science Policy Analysis
Bldg 1 Room 218D
National Institutes of Health
voice: 301-594-2567
fax: 301-402-0280

From: 孙筱放 [mailto:xiaofangsun@hotmail.com]
Sent: Wednesday, June 30, 2010 9:12 PM
To: HESCREGISTRY (NIH/NIDCD)
Subject: RE: New hESC Registry Application Request #2010-ACD-002

Dear Dr.Ellen Gadbois,

(32)

From: 筱放
To: HESCREGISTRY (NIH/NIDCD)
Subject: RE: New hESC Registry Application Request #2010-ACD-002
Date: Sunday, July 11, 2010 10:03:33 PM
Attachments: image001.png

Dear Dr. Diane Hannemann,

"Please clarify whether the IVF treatment consent and the embryo donation consent were signed at the same time."

Answer : Yes, the IVF treatment consent and the embryo donation consent were signed at the same time.] *

Thank You!

Your Sincerely,
XiaoFang Sun

From: hescregistry@mail.nih.gov
To: xiaofangsun@hotmail.com
CC: hescregistry@mail.nih.gov
Date: Fri, 9 Jul 2010 14:12:11 -0400
Subject: RE: New hESC Registry Application Request #2010-ACD-002

Dear Dr. XiaoFang Sun,

The NIH Working Group for Human Embryonic Stem Cell Eligibility Review is continuing to review this submission and is asking for additional information regarding the timing of the embryo donation process:

Please clarify whether the IVF treatment consent and the embryo donation consent were signed at the same time.

Please let us know if you have any questions about this request.

Sincerely,
-Diane Hannemann

Diane E. Hannemann, Ph.D.
Office of Science Policy Analysis
Office of the Director
Bldg 1, Room 218D
National Institutes of Health
voice: 301.594.0064
fax: 301.402.0280

PLEASE ONLY PRINT
WHEN NECESSARY.

From: 筱放 [mailto:xiaofangsun@hotmail.com]
Sent: Wednesday, June 30, 2010 9:12 PM
To: HESCREGISTRY (NIH/NIDCD)
Subject: RE: New hESC Registry Application Request #2010-ACD-002

From: 筱放
To: HESCREGISTRY (NIH/OD)
Subject: RE: New hESC Registry Application Request #2010-ACD-002
Date: Wednesday, July 21, 2010 8:29:50 PM

Dear Dr. Diane Hannemann,

1) Please confirm our understanding that the embryo donors (patients) received their clinical IVF treatment at the same institution that the stem cell derivation/research was conducted. (We note that the submitted documentation refers to both the "Third Hospital Affiliated to Guangzhou Medical College" and the "Third Hospital Affiliated to Guangzhou Medical University.")

Answer: The embryo donors (patients) received their clinical IVF treatment at the Third Hospital Affiliated to Guangzhou Medical College .There may be translation miatake.Our institution should translate into the "Third Hospital Affiliated to Guangzhou Medical College". *

2) Please explain whether you or other members of the research team had any role in the clinical IVF treatment of the embryo donors (patients).

Answer: My team members and I don't have any role in the clinical IVF treatment of the embryo donors (patients). *

3) After the review and approval of the study by the Institutional Review Board of the Third Hospital Affiliated to Guangzhou Medical University (per the October 30, 2006 approval letter), did the Institutional Review Board conduct any further review or monitoring of this study?

Answer: The Institutional Review Board of the Third Hospital Affiliated to Guangzhou Medical College did the further monitor of this study. We reported this study information to the Institutional Review Board every year. *

Thank You!

Sincerely,
XiaoFang Sun

From: hescregistry@mail.nih.gov
To: xiaofangsun@hotmail.com
CC: hescregistry@mail.nih.gov
Date: Tue, 20 Jul 2010 13:48:14 -0400
Subject: RE: New hESC Registry Application Request #2010-ACD-002

Dear Dr. XiaoFang Sun,

The Working Group for Human Embryonic Stem Cell Eligibility Review is continuing its review of this submission and has these additional questions:

1) Please confirm our understanding that the embryo donors (patients) received their clinical IVF treatment at the same institution that the stem cell derivation/research was conducted. (We note that the submitted documentation refers to both the "Third Hospital Affiliated to Guangzhou Medical College" and the "Third Hospital Affiliated to Guangzhou Medical University.")

2) Please explain whether you or other members of the research team had any role in the clinical IVF treatment of the embryo donors (patients).

3) After the review and approval of the study by the Institutional Review Board of the Third Hospital Affiliated to Guangzhou Medical University (per the October 30, 2006 approval letter), did the Institutional Review Board conduct any further review or monitoring of this study?

We appreciate your continuing efforts in this process.

Sincerely,
-Diane Hannemann

(34)

From: 筱放
To: HESCREGISTRY (NIH/OD)
Subject: RE: New hESC Registry Application Request #2010-ACD-002
Date: Thursday, July 22, 2010 10:10:49 PM
Attachments: image001.png

Dear Dr. Diane Hannemann,

I'm so greatful for your help. The response to your questions are as follows:

1. From your earlier responses, we understand that the IVF treatment and embryo donation consents were signed at the same time. Can you please tell us the approximate percentage of IVF patients that sign the embryo donation consent forms?

Answer: During 2006-2007, 1056 couples signed the embryo donation consents,but only 83 couples agreed to donate their discard embryos to stem cell research. The percentage is 7.86%(83/1056).We obtained 265 poor quality embryos from the 83 couples.

2. Upon review of the donation consent, we note that there is no language describing withdrawal of embryo donation consent. Please clarify if the donor couples were informed verbally about any ability to change their minds and withdraw consent for embryo donation.

Answer: Yes, the donor couples were informed verbally that they can change their minds and withdraw consent for embryo donation when they signed the consent.

Thank You!

Sincerely,
XiaoFang Sun

From: hescregistry@mail.nih.gov
To: xiaofangsun@hotmail.com
CC: hescregistry@mail.nih.gov
Date: Thu, 22 Jul 2010 14:56:48 -0400
Subject: RE: New hESC Registry Application Request #2010-ACD-002

Dear Dr. XiaoFang Sun,

Thank you for your quick response. This additional information is most helpful.

We hope that you may also clarify the following:

From your earlier responses, we understand that the IVF treatment and embryo donation consents were signed at the same time. Can you please tell us the approximate percentage of IVF patients that sign the embryo donation consent forms?

Upon review of the donation consent, we note that there is no language describing withdrawal of embryo donation consent. Please clarify if the donor couples were informed verbally about any ability to change their minds and withdraw consent for embryo donation.

Your responsiveness and efforts during this process is greatly appreciated. Please confirm that you have received this email.

Sincerely,
-Diane Hannemann

Diane E. Hannemann, Ph.D.
Office of Science Policy Analysis
Office of the Director
National Institutes of Health
voice: 301.594.0064
fax: 301.402.0280

35

From: 筱放
To: HESCREGISTRY (NIH/OD)
Subject: RE: New hESC Registry Application Request #2010-ACD-002
Date: Monday, July 26, 2010 2:46:46 AM
Attachments: image001.png

Dear Dr. Diane Hannemann,

During 2006.11-2007.12, 1056 couples received their clinical IVF treatment in the Third Affiliated Hospital of Guangzhou Medical College, they signed IVF treatment and embryo donation consents at the same time. Of the 1056 couples, only 83 couples agreed to donate their discard embryos to stem cell research.The percentage is 7.86%(83/1056).
Thank You!

*

Sincerely,
XiaoFang Sun

From: hescregistry@mail.nih.gov
To: xiaofangsun@hotmail.com
CC: hescregistry@mail.nih.gov
Date: Fri, 23 Jul 2010 17:24:54 -0400
Subject: RE: New hESC Registry Application Request #2010-ACD-002

Dear Dr. Xiao Fang Sun,

Thank you again for your quick response. In your first answer, you state that "1056 couples signed the embryo donation consents" and then that "83 couples agreed to donate ..." Please clarify the description of these two groups as the way it is written the two groups seem to be the same. We are hoping to understand the percentage of patients that consented for IVF treatment that then decided to donate their excess embryos to stem cell research. Please clarify if the 1056 couples is the total number of couples that agreed to undergo IVF treatment during 2006-2007.

Sincerely,
-Diane Hannemann

Diane E. Hannemann, Ph.D.
Office of Science Policy Analysis
Office of the Director
National Institutes of Health
voice: 301.594.0064
fax: 301.402.0280

PLEASE ONLY PRINT
WHEN NECESSARY.

From: 筱放 [mailto:xiaofangsun@hotmail.com]
Sent: Thursday, July 22, 2010 10:11 PM
To: HESCREGISTRY (NIH/OD)
Subject: RE: New hESC Registry Application Request #2010-ACD-002

Dear Dr. Diane Hannemann,

I'm so greatful for your help. The response to your questions are as follows:

1. From your earlier responses, we understand that the IVF treatment and embryo donation consents were signed at the same time. Can you please tell us the approximate percentage of IVF patients that sign the embryo donation consent forms?

Answer: During 2006-2007, 1056 couples signed the embryo donation consents,but only 83 couples agreed to donate their discard embryos to stem cell research. The percentage is 7.86%(83/1056).We obtained 265 poor quality embryos from the 83 couples.

(36)

From: 孙筱放
To: HESCREGISTRY (NIH/OD)
Subject: 回复: New hESC Registry Application Request #2010-ACD-002--severalquestions
Date: Tuesday, November 16, 2010 8:43:37 PM

Dear Dr. Ellen Gadbois

1.First, as you'll recall, you sent us the attached PDF with the IVF treatment consent. We had the document translated into English, which is also attached. In the English version of the Informed Consent on In Vitro Fertilization and Embryo Transfer, sentence #4 on the second page reads: "In the case of pregnancy with more than two fetuses, multifetal pregnancy reduction is necessary." Does the hospital absolutely require triplets to be reduced to twins, or is this decision left up to the patient? Have any triplets been born to parents seeking treatment at the hospital who signed the same IVF treatment conse nt form?

Answer: In the case of pregnancy with more than two fetuses, we will inform the patients that multifetal pregnancy harm to fetuses, newborn and mothers and may sesult in miscarriage and et al.. We will suggest the triplets pregnancy patients be reduced to twins after signed the treatment consent. But the descision left up to the patient , the hospital dose not absolutely require triplets to be reduced to twins.

Up to know, our hospital does not have triplets born to patients seeking treatment who signed the same IVF treatment consent form.

2.Second, please clarify that the cell line FY-3PN is not available for use by other researchers. (That is what is stated in your original submission.) Also, how would you like us to describe the karyotype of this line? We generally include that information on the Registry for lines with genetic mutations.

Answer: We would like to provide the cell line FY-3PN to other researchers. The FY-3PN cell line was established from a polyspermic embyo, the caryotype of the cell line is 69,XXX.

Thank You!
Sincerely,
XiaoFang Sun

From: hescregistry@mail.nih.gov
To: xiaofangsun@hotmail.com; stemcellresearch@yahoo.cn; xiaofangsun@hotmail.com
CC: hescregistry@mail.nih.gov
Date: Mon, 15 Nov 2010 14:04:34 -0500
Subject: RE: New hESC Registry Application Request #2010-ACD-002--several questions

Hello Dr. Sun,
Thank you for your quick response. Please be aware that the Advisory Committee to the Director will likely make a recommendation to the NIH Director on December 9 regarding whether these lines should be listed on the NIH Registry. The NIH Director, Dr. Collins, will then make a final decision sometime after the committee meeting. We will certainly let you know his decision. In the meantime, we have several more questions for you.
First, as you'll recall, you sent us the attached PDF with the IVF treatment consent. We had the document translated into English, which is also attached. In the English version of the Informed Consent on In Vitro Fertilization and Embryo Transfer, sentence #4 on the second page reads: "In the case of pregnancy with more than two fetuses, multifetal pregnancy reduction is *necessary*." Does the hospital absolutely require triplets to be reduced to twins, or is this decision left up to the patient? Have any triplets been born to parents seeking treatment at the hospital who signed the same IVF treatment conse nt form?
Second, please clarify that the cell line FY-3PN is not available for use by other researchers. (That is what is stated in your original submission.) Also, how would you like us to describe the karyotype of this line? We generally include that information on the Registry for lines with genetic mutations.
Please let us know if you have any questions about this request.
Sincerely,
Ellen Gadbois
Ellen L. Gadbois, Ph.D.
Office of Science Policy Analysis
Bldg 1 Room 218D
National Institutes of Health
voice: 301-594-2567
fax: 301-402-0280

From: 孙筱放 [mailto:xiaofangsun@hotmail.com]
Sent: Wednesday, November 10, 2010 7:56 PM
To: HESCREGISTRY (NIH/OD)
Subject: FW: New hESC Registry Application Request #2010-ACD-002
Dear Dr. Diane Hannemann
Thank you for your help with our hESC Registry. There is no commercial harm to me if the information I have indicated is