Stem Cell Research 48 (2020) 101956

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Pluripotent stem cell regulation in Spain and the Spanish National Stem Cell Bank

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A B S T R A C T

The Spanish National Stem Cell Bank (Banco Nacional de Líneas Celulares, BNLC) was established in 2006 thanks to a change in the legislative framework in Spain. The Law 14/2006 updated the previous Assisted Reproduction Techniques Law (Law 45/2003) allowing the use of the surplus frozen embryos following IVF for research. The BNLC has a network structure with 3 nodes: the Regenerative Medicine Program (IDIBELL), the Principe Felipe Research Center (CIPF) in Valencia and the Andalusian Public Health System Biobank (SSPA Biobank) in Granada. The aim of the BNLC is to guarantee throughout the national territory the availability of human stem cell lines for biomedical research. At present time, there are 40 human embryonic stem cell lines (hESC) and 171 human induced pluripotent stem cell lines (hiPSC) registered in the BNLC. These lines are fully characterized and available in the context of research projects approved by the Technical Committee of the BNLC.

1. Introduction

The Spanish National Stem Cell Bank (*Banco Nacional de Líneas Celulares, BNLC*) was established in 2006 thanks to a change in the legislative framework in Spain. The Law 14/2006 updated the previous Assisted Reproduction Techniques Law (Law 45/2003) allowing the use of the surplus frozen embryos following IVF for research. Any couple with frozen embryos, regardless of the time of cryopreservation, can decide on the future of their embryos from among four options: (i) use by the couple for reproduction; (ii) anonymous donation to other couples; (iii) donation for research purposes; and (iv) disposal without further use. The new legislation allowed couples to keep embryos frozen throughout the woman's reproductive life without establishing time limits. Following this legislation, the Spanish Law on Biomedical Research, 14/2007 was established. This law forbids the creation of human embryos for research purposes but allows the use of surplus frozen embryos for human embryonic stem cells (hESC) and reprogramming technologies such as nuclear transfer or the generation of human induced pluripotent stem cells (hiPSC).

As a consequence, of this legislation the General Subdirectorate for Research in Cell Therapy and Regenerative Medicine of the Instituto de Salud Carlos III was created with the aim to promote stem cell research and regenerative medicine. It was also tasked with the founding of the National Stem Cell Bank (NSCB). The BNLC has a network structure with 3 nodes: the Regenerative Medicine Program (IDIBELL), the Principe Felipe Research Center (CIPF) in Valencia and the Andalusian Public Health System Biobank (SSPA Biobank) in Granada. The aim of the BNLC is to guarantee throughout the national territory the availability of human stem cell lines for biomedical research. Any hPSC must be generated in the context of a research project that needs to be evaluated by the "*Comisión de Garantías para la Donación y Utilización de Células y Tejidos Humanos*" created also as a consequence of the Law 14/2007. This framework was established to ensure that the hESC and hiPSC generation and research are conducted according to the legal, ethical and scientific guidelines. A local Ethics Committee authorization of the project is also needed. It is mandatory to register, deposit and bank all human pluripotent cell lines (hPSC) generated in Spain and make them available to the researchers nationwide.

The BNLC is partially funded by the "*Convocatoria de Plataformas de Apoyo a la Investigación en Ciencias y Tecnologías de la Salud*" (Plataforma de Proteómica, Genotipado y Líneas celulares, PRB3) from the Subdirectorate General of Evaluation and Promotion of Research of the Instituto de Salud Carlos III.

2. Description of cell lines distributed and conditions of supply

At present time (June 2020), there are 40 hESC lines and 171 hiPSC lines currently registered in the BNLC. Among available hESC lines: 21 come from healthy embryos, 2 from parthenogenetic embryos, 3 from single blastomeres and 2 from PGD (preimplantation genetic diagnosis) discarded embryos carrying genetic mutations (multiple exostosis and myotonic dystrophy). Twelve lines were generated by the genetic

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https://doi.org/10.1016/j.scr.2020.101956
Received 27 May 2019; Received in revised form 22 June 2020; Accepted 26 July 2020
Available online 24 August 2020

modification of already existing lines. One hundred-seventy-one hiPSC lines are currently registered in the BNLC. Most of them have been generated by retroviral (68) and Sendai virus infection (78), but there are also lines reprogrammed by lentivirus (12), episomal factors (10) or mRNA (3). Fibroblasts were used as original cells in 121 lines, but also cord blood (16), keratinocytes (9), peripheral blood mononuclear cells (15), urine cells (1), adipose derived mesenchymal stem cells (2), Schwann cells (6) or fetal hepatocytes (1) served as somatic cell lines. These cells were donated by healthy subjects and patients suffering from 39 different diseases such as Parkinson's disease, multiple sclerosis, autism, neurofibromatosis and others. These lines were generated in more than 20 different research centres.

Pluripotent stem cell lines can be accessed from the BNLC in the context of a research project that needs to be approved by the Technical Committee of the BNLC. The application to access the lines must be accompanied by detailed information about the research project that must be evaluated by the "*Comisión de Garantias para la Donación y Utilización de Células y Tejidos Humanos*". A local Ethics Committee authorization of the project is also needed and a MTA (Material Transfer Agreement) must be signed between the researcher and the BNLC. The cession (vial release/supply) is free and only expansion and maintenance costs are charged.

3. Summary of tests for QC, characterisation and safety performed on each distributed cell line

The minimum requirements requested for characterization and QC are:

- 1 Pluripotency:
  a. Alkaline phosphatase test
  b. Pluripotency markers detection (by immunocytochemistry, flow cytometry or PCR)

  Oct4, Nanog, Sox2, SSEA3, SSEA4, Tra-1-60, Tra-1-81.

- 2. Karyotype.
- 3. Evidence of absence of reprogramming transgenes.
- 4. In vitro differentiation

  three germ layer detection (endoderm, mesoderm and ectoderm) by embryoid bodies formation. One marker minimum for germ layer.

- 5 In vivo differentiation

  three germ layer detection (endoderm, mesoderm and ectoderm) by teratomas induction in SCID mice. One marker minimum for germ layer.

- 6. Mycoplasma testing
- 7. DNA fingerprinting study in the hPSC line and in the original cells.
- 8. Confirmation of genotypic diagnosis of the cell line generated from samples with genetic mutation.

4. Information provided with cell lines

Information about registered cell lines at the BNLC web (http://www.isciii.es/ISCIII/es/contenidos/fd-el-instituto/fd-organizacion/fd-estructura-directiva/fd-subdireccion-general-investigacion-terapia-celular-medicina-regenerativa/fd-centros-unidades/fd-banco-nacional-lineas-celulares/lineas-celulares-disponibles.shtml). In addition to the results of the characterization, information regarding donors and methodological details are provided:

- Donor: source of original cells, age, sex, disease, mutation, fingerprinting.
- Methodology: original cells and hiPSC culture conditions, reprogramming methodology, freezing and thawing method.

5. Other services and training provided to the outside community

Technical support for hPSC generation and characterization is offered and even the possibility of training in handling and culture of hPSC in the facilities of the nodes. Courses and workshops are periodically organized. In addition, advice and management on ethical, legal and administrative issues of research projects focused on pluripotent cells are available.

6. Any accreditation for regulatory and/or ethical issues

Any hPSC must be generated in the context of a research project authorized by the competent state or autonomous region's authority, following a favourable report from the Guarantees Committee for the Donation and Use of Human Cells and Tissues of the BNLC. An Ethics Committee authorization is also needed. The research will have been carried out complying with the ethical principles and the applicable legal regime, especially the provisions of the Biomedical Research Law. The applicant institution will keep the records identifying the donor subjects strictly confidential, in accordance to the provisions of the Organic Law 15/1999 of December 13 on the Protection of Personal Data, and it is necessary that the donors have been informed about this law. Applications for the cell line deposition to the BNLC, both for hESC and hiPSC, must be authorized by the legal representative of the institution where the lines have been derived.

7. Future plans

The current objectives of the BNLC are:
Services (In addition to those mentioned previously):

- Optimization and set up of differentiation protocols as a service available to researchers.
- Development and supply of culture media stocks in collaboration with companies working in the area of PSC field.

Research:

- Generation of hiPSC lines from patients to be used as disease models for basic and clinical research groups.
- Collaboration with international banks and registries (EBISC, hPSCreg, GAiT, ISCBI, UK Stem Cell Bank).
- Creation of a registry of pre-clinical and clinical trials with hPSC –derived cells.
- Creation of a bank of hiPSC from cord blood cells in homozygosis for HLA-A, HLA-B and HLA- DRB1 antigens generated in GMP conditions.
- Creation of a database of hPSC genotyping.
- Development of new hPSC generation and culture methodologies.

Training:

- Organization of workshops and courses covering hPSC generation, culture and characterization.
- Training of PhD and master students in the handling of hPSC.

Dissemination:

- Development and improvement of the BNLC web.
- Development of a knowledge dissemination plan amongst basic and clinical researchers involving Social networks.
- Promotion of the hPSC registry amongst basic and clinical

researchers.
- Promotion of hPSC publications in national and international journals.

Acknowledgments

The National Spanish Stem Cell Bank is supported by the Plataforma de Proteomica, Genotipado y Líneas Celulares (PT1770019/0015) (PRB3), Instituto de Salud Carlos III.