Article

Human embryonic stem cell lines with genetic disorders

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Abstract

A previous study described the establishment of human embryonic stem cell (ESC) lines from different sources of embryonic material, including morula, whole blastocyst and isolated inner cell mass. Using these methods, a repository of ESC lines has been established with different genetic abnormalities, which provides an unlimited source of disease cells in culture for undertaking research on the primary disturbances of the cellular processes in the genetically abnormal cells. ESC lines with genetic disorders were derived from the mutant embryos detected and avoided from transfer in the ongoing practice of preimplantation genetic diagnosis (PGD). The current repository contains 18 ESC lines with genetic disorders, including adrenoleukodystrophy, Duchenne and Becker muscular dystrophy, Fanconi anaemia, complementation group A, fragile-X syndrome, Huntington disease (three lines), Marfan syndrome, myotonic dystrophy (two lines), neurofibromatosis type I (five lines) and thalassaemia (two lines). These ESC lines are presently used for research purposes and may be available on request.

Keywords: *embryonic stem cells (ESC), ESC lines with genetic disorders, preimplantation genetic diagnosis, repository of human ESC lines, single gene disorders*

Introduction

A new method has been described for the derivation of human embryonic stem cell (ESC) lines from morula, and a repository of normal ESC lines from different sources of embryonic material has been established, including morula, whole blastocyst and isolated inner cell mass (Strelchenko *et al*., 2004). These lines were characterized by a set of ESC criteria, including the presence of Oct-4, TRA-2–39, high molecular weight glycoproteins (antibodies TRA-1–60, TRA-1–80), stage-specific embryonic antigens (SSEA-3, SSEA-4), and euploid karyotype.

These developments made it possible to initiate the establishment of a repository of ESC lines from embryos with different genetic abnormalities, which will have an important role in the study of the primary disturbances of the cellular processes in the mutant cells to identify the molecular mechanisms that might be blocked to prevent the disease progression. Therefore, there is an obvious need for establishment of ESC lines derived from embryos with genetic and chromosomal abnormalities, to provide the basis for understanding of the mechanisms of the phenotypic realization of genetic defects and for the development of new approaches for their possible treatment.

The establishment of ES cell lines with genetic disorders has become possible with the introduction of preimplantation genetic diagnosis (PGD), enabling physicians to avoid transfer of the affected embryos, which then provide a valuable source for ESC lines with genetic abnormalities. This also provides a unique opportunity for investigating the potential of establishing ESC lines depending on the genotype. This paper presents the first description of the repository of human ESC lines with genetic disorders.

Materials and methods

The preimplantation embryos for the establishment of ESC lines with genetic disorders were obtained from PGD cycles, which

were performed either by the first and second polar body (PB1 and PB2) removal or embryo biopsy, as described elsewhere (Verlinsky and Kuliev, 2000). Following multiplex PCR analysis of either PB1 and PB2, or single blastomeres (Rechitsky *et al*., 2001, 2002), the unaffected embryos were transferred back to patients, while the mutant ones were used for confirmation of PGD or donated for research, according to informed consent approved by the IRB of Reproductive Genetics Institute. In some cases, the embryos were obtained after PGD combined with HLA typing, as described elsewhere (Rechitsky *et al*., 2004).

Depending on the developmental stage of these donated mutant embryos, different techniques for the establishment of ESC lines were used, as described previously (Strelchenko *et al*., 2004). The initial disaggregation of the cells (passage 0) was performed approximately 8–14 days after growth in feeder layer, by treating the cells with EDTA and cutting and transferring the soft cell clumps into a new dish with feeder layer. Fast proliferating colonies with ES-like morphology were isolated and propagated further. Within the next two to five passages, the uniform proliferating cells were selected, and colonies of established ESC lines were passaged using EDTA, followed by the harvesting procedure with a cell lifter, as described previously (Strelchenko *et al*., 2004).

The cell lines were tested for the following ES cell markers: alkaline phosphatase, stage-specific antigens SSEA-3 and SSEA-4, high molecular weight glycoproteins TRA-1–60 and TRA-1–80, and Oct-4, detected with polyclonal antibodies, as well as by Gene Choice One Tube RT-PCR kit (Vector Laboratories Inc., Burlingame, CA, USA), as described previously (Strelchenko *et al*., 2004).

The established human ES-cell lines were maintained *in vitro* from 10 to 15 passages before freezing in sufficient amounts.

Results

The list of 18 ESC lines obtained from the embryos with genetic disorders is presented in **Table 1**. This repository contains three ESC lines with autosomal recessive disorders, including thalassaemia and Fanconi anaemia, complementation group A (FANCA), four ESC lines with X-linked disorders, including adrenoreukodystrophy (ABCD1), fragile site mental retardation (FMR1), Duchenne and Becker type muscular dystrophy (DMD; BMD), and 11 ESC lines with autosomal dominant conditions, including five ESC lines with dynamic mutations.

Only three ESC lines of the repository represent unaffected carriers, including one ESC line with IVSI-110 beta-thalassaemia mutation, one with a 14 bp deletion in FANCA, and one with DMD. The remaining 15 ESC lines were affected. One of the ESC lines with beta-thalassaemia has a double heterozygous genotype (Cd 39/IVSI-110), with an IVSI-110 mutation inherited from the mother, and Cd 39 inherited from the father. As mentioned above, the largest group of ESC lines was obtained from PGD for dominant mutations, which, in addition to five ESC lines with dynamic mutations, contains five ESC lines with neurofibromatosis type 1 (NF1), obtained from NF1 embryos detected in PGD for NF1, described earlier (Rechitsky *et al*., 2002; Verlinsky *et al*. 2002). While 17 ESC lines were derived from PGD practice, one ESC line (DMD) was established by request of a couple, and originated from the cohort of spare donated embryos, the transfer of two of which resulted in the birth of DMD twins.

Table 1 presents the results of PGD for only those conditions with which the above ESC lines were obtained. As seen from the data, the unaffected embryos were selected for transfer in all but two PGD cycles, with the affected embryos available as a source for the establishment of ESC lines with genetic disorders in each of the cycles shown in **Table 1**.

Figure 1 presents the results of PGD cycles resulting in the establishment of ESC line with Huntington disease (HD), a severe late onset autosomal dominant neurodegenerative disorder, which is one of the common indications for PGD. As can be seen from the presented pedigree, because of the female partner being a carrier of exon 1 expansion of the HD gene located on chromosome 4p13.3, PGD was performed using PB1 and PB2 analysis, which predicted eight oocytes with the expanded allele and nine mutation-free oocytes. Two embryos resulting from these mutation-free oocytes (oocytes 5 and 10) were transferred, with the remaining mutation-free ones that reached blastocyst stage being frozen for future use by the couple. Three of eight mutant embryos were donated for research and used for establishment of ESC lines, resulting in two ESC lines, which apparently originated from the oocytes 12 and 17, as demonstrated by linked marker analysis, also confirming the presence of the expanded allele in exon 1 (hESC–186 and hESC–194). In addition, another ESC line with HD was obtained from PGD for the paternally derived mutation in the other PGD cycle and was also confirmed to contain the paternal expanded allele (**Table 1**).

Figure 2 presents the results of PGD for adrenoleukodystrophy (ALD) performed in combination with HLA typing, resulting in the establishment of ESC with this condition. The couple had two previous children affected with ALD (one of whom died), caused by the 1801 deletion of AG of *ABCD1* gene located in the X-chromosome (Xq28), inherited from the mother. Because the mutation leads to a progressive multifocal demyelination of central nervous system with adrenocortical insufficiency in boys, effectively treated only by HLA-compatible stem cell transplantation, the couple requested PGD combined with preimplantation HLA typing. PGD was performed by blastomere biopsy, which predicted one unaffected male, one unaffected female and one affected male embryo, which was the only one that was HLA-matched to the affected sibling. This embryo was donated for research and used for the establishment of ESC line, confirmed to contain an 1801 deletion of AG in the *ABCD1* gene.

The other ESC line obtained from PGD combined with HLA typing was the ESC line with double heterozygous thalassaemia, which was performed by blastomere biopsy and multiplex PCR analysis, involving simultaneous testing of both mutation and HLA markers (Rechitsky *et al*., 2004). Of 10 embryos tested, three were affected, one contained only one chromosome 11 with codon 39 mutation and the remaining six were carriers of one of the mutations tested. Only one of these embryos was a full HLA match and was transferred. Of the six affected embryos, one embryo that was also an HLA non-match was donated for research and resulted in the establishment of an ESC line. The follow-up testing showed that the cells were double heterozygous affected for Cd39 and IVSI-110 mutations. As mentioned,