Chapter 14
PGD and Human Embryonic Stem Cell Technology

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T. El-Toukhy, P. Braude (eds.), *Preimplantation Genetic Diagnosis in Clinical Practice*, DOI 10.1007/978-1-4471-2948-6_14,
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Human embryonic stem (hES) cells are derived from an early embryo and can grow in vitro indefinitely, while retaining their capability to differentiate into specialised somatic cell types. Two types of hES cells are being derived:

1. Those intended for clinical use in regenerative medicine
2. Those that carry disease-specific mutations and may serve as ‘disease-in-a-dish’ models

PGD technology was instrumental in the derivation of both types of hES cells.

Derivation of hES Cell Lines

The first reported hES cells were derived from blastocysts generated by in vitro fertilisation at the University of Wisconsin. By the end of 2009, nearly 1,200 original hES cell lines had been reported from 24 countries. Most of the lines were derived from the inner cell mass (ICM) of blastocyst stage embryos, employing a technology similar to the one developed by the group from Wisconsin. Reports that hES cells could be derived from the late morula/compaction stage and from cleavage stage embryos arrested in development demonstrated that pluripotent cells could give rise to hES cell lines at any stage of early embryo development.

hES cell culture is generally made up of two basic components – a fibroblast ‘feeder’ layer to which the hES cells attach and a specially designed culture medium which surrounds the cells as they grow. Within a stable temperature and gaseous environment, these components provide the cells with a scaffold to grow over and nutrients for growth, maintain an appropriate pH and, when optimal, support rapid undifferentiated proliferation. The exact details of these components vary across laboratories around the world, and differing levels of success are achieved in terms of derivation rates and cell line maintenance efficiency. The original culture systems used murine feeder cells and media based around fetal bovine serum. Most of the known hES cell lines

have therefore been derived under conditions that expose the cells to products of animal origin, which renders them unsuitable for clinical use due to the risk of transmitting animal pathogens to recipients. Over time, however, the replacement of murine with human feeders and media based on bovine with defined culture media devoid of animal products has led to xeno-free derivation and expansion techniques for both research and clinical grade hES cell lines. As well as xeno-free derivation and propagation, stringent record-keeping, proof of sterility, negative virology and detailed characterisation of the cell lines are necessary to meet current clinical grade recommendations and good manufacturing practice (GMP) criteria, qualifying the cells for therapeutic use. Characterisation of the cells routinely includes DNA fingerprinting, HLA typing, karyotyping, expression of pluripotency markers and differentiation into derivatives of the three germ layers with in vitro expression of markers and in vivo teratoma growth.

Derivation of hES Cell Lines from a Single Blastomere

To avoid ethical and political controversies surrounding use of supernumerary embryos in research, efforts have been made to use alternative methods such as somatic cell nuclear transfer and parthenogenetic activation of human oocytes. However, neither of them has become a mainstream approach. The method that has become a popular option for circumventing political restrictions, particularly in the USA, is the derivation of hES cell lines from single cells of the cleavage stage embryo. Robert Lanza's group from the American company Advanced Cell Technology from Massachusetts (www.advancedcell.com) utilised a PGD technique to develop a groundbreaking approach to the derivation of hES cell lines using a single biopsied blastomere. Although the embryo was destroyed in the process, this was proof of principle that a hES cell line could be derived without embryo

destruction. Indeed, in subsequent reports from the same group as well as others, the biopsied embryos survived and developed to the blastocyst stage. hES cell lines derived from a single blastomere exhibit very similar transcriptional profiles to hES cell lines derived from the ICM, suggesting that over time in culture hES cells acquire virtually identical stable phenotypes and are not affected by the developmental stage of the starting cell population.

One of the lines developed by Lanza's group from a single blastomere, MA-09, was used to develop an hES cell-based therapy, currently in clinical trials in multiple centres in the USA and UK, for the treatment of Stargardt's macular dystrophy and dry age-related macular degeneration.

Derivation of Specific Mutation-Carrying hES Cell Lines

Embryos diagnosed as being genetically unsuitable for replacement following PGD analysis would routinely be discarded, despite often being of good quality and capable of developing into blastocysts. With appropriate consent, these blastocysts are suitable for stem cell derivation and are free from many ethical difficulties associated with using normal embryos from patients seeking infertility treatment. With the fundamental attributes of pluripotency and self-renewal, hES cells carrying genetic disorders hold promise for an unlimited supply of cells with which to study the mechanisms and development of the disease. hES cells from PGD embryos should represent an even more relevant model than genetically altered cells or animal models as the mutant protein is expressed in its normal physiological context and range of expression pattern. The use of such 'disease-in-a-dish' models of genetic and degenerative disorders would also reduce the need for animal models. If highly purified colonies of known constituent cells can be developed through precise characterisation and sorting, they may prove useful in the long run, especially in terms of drug screening for new compounds and

testing of unacceptable side effects. This could speed up the development to implementation process thus getting new drugs out more rapidly for use by the public. hES cell lines, particularly those carrying clinically relevant mutations, have gained considerable interest from the biopharmaceutical sector. The pharmaceutical discovery process is generally accepted as being time consuming and inefficient requiring high levels of financial investment without any guarantee of a clinical product at the end. Improvements to the discovery phase of new compounds could come through the development of more tailored, disease-orientated cellular screens, for both therapeutic target validation and optimisation of identified compounds.

hES cell lines have been derived with a number of monogenic disorders as well as disease-specific translocations (Tables 14.1 and 14.2). The largest number of PGD cell lines have been derived from embryos carrying a mutation for cystic fibrosis (22) and Huntington's disease (20). We have previously published our experience with the use of affected PGD embryos having derived the first hES cell line in the UK and the first line with a cystic fibrosis mutation. In addition we have now derived more than 20 hES cell lines carrying clinically relevant genetic mutations for eight monogenic diseases and one translocation (Table 14.3). The validation of hES cells as models of disease has begun for some disorders, including Huntington's disease, myotonic dystrophy and fragile X.

Induced Pluripotent Stem Cell Lines

In 2006, scientists found a way of reverting differentiated cells from adult mice into an embryonic-like state. In a groundbreaking publication, the following year Yamanaka and colleagues described the induction of pluripotent stem (iPS) cells from adult human fibroblasts by defined factors that are associated with the pluripotent state. These iPS cells were similar (but not identical) to hES cells in morphology,

TABLE 14.1 Specific mutation-carrying hES cell lines reported by May 17, 2012

Disease	Number of lines
Adrenoleukodystrophy	1
Albinism ocular, type1	2
Alpha-thalassaemia	2
Alport syndrome	2
Beta thalassaemia	6
Beta thalassaemia carrier	3
Breast cancer	2
Breast cancer and endocrine neoplasia	1
Charcot-Marie-Tooth disease, type 1A	3
Charcot-Marie-Tooth disease, type 1B	1
Cystic fibrosis	22
Cystic fibrosis carrier	1
Epidermolysis bullosa	1
Fabry syndrome	1
Fanconi anaemia – a carrier	1
Fragile site mental retardation 1, carrier	1
Fragile X syndrome	6
Fragile X syndrome, carrier	5
Gaucher disease	1
Haemoglobin alpha locus	1
Haemoglobin beta locus mutation	3
Haemophilia A	1
Huntington's disease	20
Huntington's disease and Marfan syndrome	1
Hypochondroplasia	1
Incontinentia pigmenti	1
Infantile neuroaxonal dystrophy	1
Juvenile retinoschisis	1

TABLE 14.1 (continued)

Disease	Number of lines
Marfan syndrome	3
Merosin-deficient congenital muscular dystrophy, type 1A	1
Multiple endocrine neoplasia, type 1	1
Multiple endocrine neoplasia, type 2	3
Muscular dystrophy, Becker	1
Muscular dystrophy, Becker, carrier	1
Muscular dystrophy, Duchenne	5
Muscular dystrophy, Duchenne, carrier	1
Muscular dystrophy, Emery-Dreifuss	1
Muscular dystrophy, Emery-Dreifuss, carrier	3
Muscular dystrophy, facioscapulohumeral	9
Muscular dystrophy, facioscapulohumeral and Turner syndrome	1
Muscular dystrophy, facioscapulohumeral, putative	2
Myotonic dystrophy	6
Myotonic dystrophy, type 1	4
Myotonic dystrophy, type 2	1
Nemaline myopathy 2	2
NEMO deficiency	2
Neurofibromatosis, type I	9
Osteogenesis imperfecta, type 1	1
Patau syndrome	1
Pelizaeus-Merzbacher disease	1
Popliteal pterygium syndrome	1
Saethre-Chotzen syndrome	1
Sandhoff disease	1
Sickle-cell anaemia	2

(continued)

TABLE 14.1 (continued)

Disease	Number of lines
Spinal muscular atrophy, type I	3
Spinocerebellar ataxia, type 2	1
Spinocerebellar ataxia, type 7	1
Torsion dystonia	1
Torsion dystonia 1	4
Translocation, 7:12	1
Translocation, 7:17	1
Translocation, 11:22	1
Treacher Collins-Franceschetti syndrome	2
Tuberous sclerosis, type 1	3
Turner syndrome, mosaic cell line	1
Vitelliform macular dystrophy	2
Von Hippel-Lindau disease	5
Wilms' tumour	1
Wiskott-Aldrich syndrome, cystic fibrosis carrier	1
X-linked myotubular myopathy	2
Zellweger syndrome	1

Strulovici et al. (2007), Löser et al. (2010); University of Massachusetts Medical School, International Stem Cell Registry http://www.umassmed.edu/iscr/GeneticDisorders.aspx; http://www.stemride.com/ accessed on May 17, 2012, including those derived at Assisted Conception Unit at Guy's Hospital as of May 17, 2012, sorted by disease type

proliferation, surface antigens, gene expression and differentiation ability. These cells are therefore as powerful as those isolated from early embryos and free of controversy. Since then, this new iPS cell field has enjoyed unprecedented popularity. Indeed all the advantages of using PGD hES cell lines as disease models also apply to the use of human iPS cells with the added attraction of a greater number of diseases,

Table 14.2 Specific mutation-carrying hES cell lines reported by May 17, 2012

Country	Number of lines
USA	91
Australia	32
UK	22
France	21
Israel	18
Belgium	16
Spain	1
Turkey	1
Total	202

Strulovici et al. (2007), Löser et al. (2010); University of Massachusetts Medical School, International Stem Cell Registry http://www.umassmed.edu/iscr/GeneticDisorders.aspx; http://www.stemride.com/ accessed on May 17, 2012, including those derived at Assisted Conception Unit at Guy's Hospital as of May 17, 2012, sorted by country of derivation

Table 14.3 Specific mutation-carrying hES cell lines derived at Assisted Conception Unit at Guy's Hospital as of May 17, 2012

Disease	Number of lines
Beta thalassaemia	1
Beta thalassaemia carrier	1
Cystic fibrosis	4
Huntington's disease	7
Myotonic dystrophy, type 1	1
Neurofibromatosis, type I	2
Spinal muscular atrophy, type I	1
Translocation, 7:12	1
Von Hippel-Lindau disease	3
Wiskott-Aldrich syndrome, cystic fibrosis carrier	1

easier availability of starting material, the use of samples from the diseased tissue and from donors in the age range when the disease occurs and fewer ethical issues. Furthermore stem cell models of some complex degenerative diseases such as Parkinson's, autism or Alzheimer's, for which no single predictive gene has been identified, will not be available through PGD but could be through the use of iPS technology.

Future Outlook

hES cells remain the only genetically unmodified pluripotent cells and as such remain the gold standard for pluripotency research. Research into the field of hES cells will continue in order to understand the basic mechanisms of pluripotency and self-renewal, as the gold standard with which to compare iPS cells; to investigate heterogeneity in pluripotent cells, as a powerful tool for modelling diseases; to investigate early human development; and because hES cells are years ahead of iPS cells in terms of safety for preclinical and clinical studies. Therefore the importance of PGD as a source of embryos for stem cell research is likely to grow. With the ever-increasing number of diseases for which PGD can be offered, and the continuous improvements to hES cell derivation and propagation methods, we anticipate that our bank of mutation-carrying hES cell lines will continue to grow rapidly, providing a unique and vital cell source that is freely available to researchers worldwide.

Key Points

- PGD technology has been instrumental in the derivation of both research and clinical grade human embryonic stem (hES) cell line.
- A line derived using the PGD technique of single blastomere biopsy is being used in the first clinical trial in Europe using hES cells and is the only currently ongoing clinical trial in the world.

- hES cell lines carrying clinically relevant genetic mutations are derived from affected embryos diagnosed in a PGD cycle and can be used as 'disease-in-a-dish' models.
- These PGD lines, in combination with induced pluripotent stem cell lines from affected patients, are a powerful tool for disease research.
- There is interest from the biopharmaceutical sector in the use of these mutation-carrying lines for drug discovery and toxicology screening.

Further Reading

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