The International Stem Cell Initiative: toward benchmarks for human embryonic stem cell research

The Steering Committee of the International Stem Cell Initiative*

An international consortium is comparing the properties of 75 human embryonic stem cell lines.

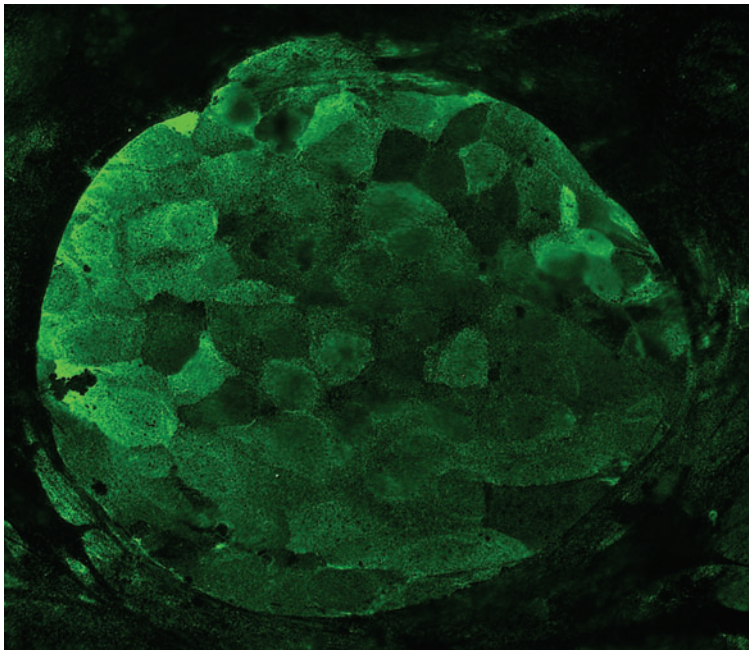
Since the first report of the derivation of human embryonic stem (hES) cells in 1998[1], the number of research groups working on this unique and versatile type of cultured cell has expanded rapidly[2,3]. The original observation that pluripotent stem cells could be derived at reasonable frequency from the human preimplantation embryo has been widely and repeatedly confirmed, to the extent that the scientific literature now contains reports of the isolation of over 100 hES cell lines. Although most researchers have performed immunological, molecular and biological characterization to determine stem cell phenotype and differentiation capacity, only a few lines have been examined in any real depth by more than one laboratory, and little systematic comparison of cell lines has been carried out.

It is critical for progress in the field to understand the similarities and the differences between the various isolates, so that research results from different laboratories can be compared in a meaningful fashion. For example, a recent flurry of papers has described a variety of new culture systems for the maintenance of hES cells *in vitro*. These studies potentially represent important technical advances for the field. But without knowing how much particular cell lines differ under a given set of growth conditions, or how the properties of a given cell line vary when grown in different laboratories, it is difficult to assess the generality of these results.

Studies of the hES cell transcriptome also raise issues of how similar or dissimilar various lines may be. In recent years the canonical description of the hES cell phenotype has been greatly expanded by microarray and other transcriptional profiling analyses, some of which provide an in-depth assessment of hES cell line heterogeneity. Certain studies[4–8] have reported similarities in overall patterns of gene expression between cell lines, whereas others have emphasized differences[9,10]. These reports have examined relatively limited numbers of stem cell lines and have not provided much in the way of interlaboratory comparison. If hES cell lines actually do vary in their growth and differentiation properties, some may prove far more suitable for specific applications in research or regenerative medicine than others.

Origins of the Initiative

The International Stem Cell Forum (http://www.stemcellforum.org.uk), founded in January 2003 and chaired by the Medical Research Council of the United Kingdom, is a group made up of representatives of medical research funding bodies from 15 countries with the goal of promoting international



Colony of hES cells stained with antibody GCTM-2.

Authors and their affiliations are listed at the end of the paper.

collaboration and funding support for stem cell research (**Table 1**). The International Stem Cell Initiative grew out of a meeting held under the auspices of the Forum in London in May 2003. The meeting brought together experts in hES cell research from around the world to plan an international collaborative effort to establish a set of standards for the characterization of hES cell lines.

The group decided to begin with what was envisioned to be a relatively simple project, namely, to collect as many hES cell lines as possible and carry out a basic set of characterization studies on them under defined conditions. The exercise, which is supported by funding from the Forum members, is being conducted with the cooperation of the UK Stem Cell Bank as a central hub for collection and distribution of materials. Forum members were invited to nominate laboratories to submit their hES cell lines to the Initiative. Prospective participating laboratories were asked to certify that their hES cell lines had been derived following generally accepted ethical guidelines and to agree that all information generated by the Initiative would be placed in the public domain. Seventeen laboratories from 11 Forum member countries agreed to participate and are contributing a total of 75 hES cell lines to the study (**Fig. 1**). These laboratories are carrying out surface-antigen expression analyses on their own cells and preparing nucleic acids and other samples for study by several other central reference laboratories.

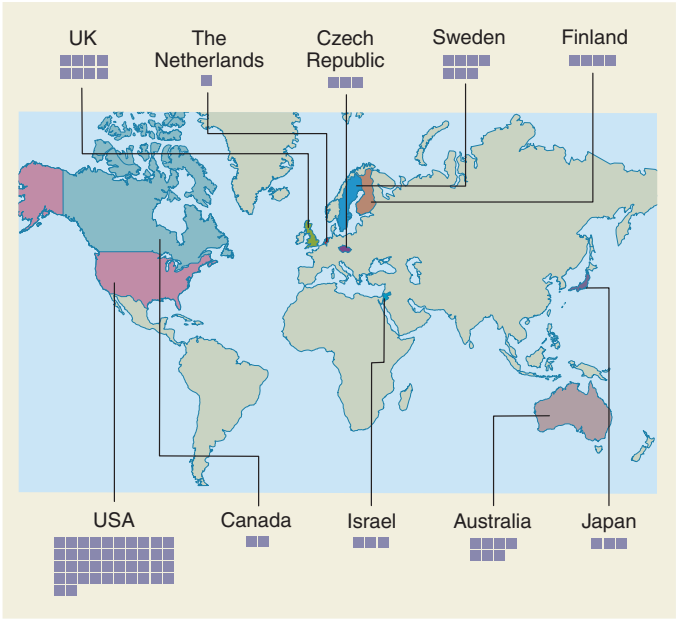


Figure 1 Countries of origin of hES cell lines in the Initiative. Blocks indicate number of hES cell lines contributed by each country.

Characterization studies

The studies include flow cytometric analysis of the expression of 17 surface antigens, quantitative RT-PCR analysis of the transcript levels of ~100 genes characteristic of pluripotent stem cells and their early differentiated derivatives, and an examination of how the expression pattern of these ~100 genes changes in response to a simple differentiation protocol involving embryoid body formation. The antigens chosen are those commonly used by many groups to define hES cells. They include markers such as SSEA3, SSEA4, *THY1* and the antigens defined by antibodies TRA-1-60 and GCTM2, all of which have been previously reported to be characteristically expressed by undifferentiated hES cells. To ensure standardization, agreement was reached with the owners of all the key hybridomas that define these marker antigens to deposit them in an archive at the National Institute of Biological Standards and Control in the UK, the home of the UK Stem Cell Bank and a WHO Reference Laboratory.

The gene expression studies are focused on molecules that are widely reported to be good markers of human pluripotent stem cells, including some whose functions are likely essential to maintenance of pluripotentiality, such as *POU5F1* (also known as *OCT4*), *NANOG*, *SOX2*, *ZFP42* (also known as *REX1*), *UTF1*, *GDF3*, *FOXD3*, *TERT*, *FGF4*, and others, such as *LIFR* and *LRPPRC* (also known as *GP130*), whose role in maintaining pluripotentiality is more controversial. Also included in the analysis are genes whose expression marks particular differentiation lineages, for example, *T* (also known as *BRACHYURY*; mesoderm), *MYF5* and *MYOD1* (muscle markers), *GATA4* (endoderm), *TAT* (hepatocytes), and *INS* (pancreatic beta cells).

Additional studies are aimed at assessment of the epigenetic status of the cell lines (expression of imprinted genes), examination of spatial patterns of marker expression in growing colonies by immunostaining *in situ*, and histological evaluation of teratomas formed by the cell lines. In addition, each line will be subjected to DNA fingerprinting, to provide definitive markers for identifying each line in future studies, and to microbiological analysis that will include a screen for possible endogenous retrovirus expression. Karyotyping will not be performed, but participants will be asked to provide karyotype data for each of their lines. Likewise, although the Initiative will not examine xenograft tumor production, participating laboratories have been invited to submit histological slides of any xenografts that they have produced from their lines for review by a histopathologist with expertise in this area.

The first examination of the preliminary dataset will take place at a two-day meeting of the Initiative participants at the Jackson Laboratory, in Bar Harbor, Maine, in August 2005. The entire analysis should be completed by the end of 2005. All the data will be placed in the public domain and will be available from the Forum website.

Goals of the Initiative

What are the expected outcomes of this first phase of the Initiative? Most researchers anticipate that expression of canonical cell-surface markers and pluripotency genes will be fairly consistent across the panel of cell lines, but in fact an exercise on this scale may turn up outliers with highly informative properties.

Table 1 Members of the International Stem Cell Forum

Countries	
Australia[a]	Japan[a]
Canada[a]	Netherlands[a]
Czech Republic[a]	Singapore[a]
Denmark	Sweden[a]
France	Switzerland
Germany	UK[a]
Finland[a]	USA[a]
Israel[a]	
International member	
Juvenile Diabetes Research Foundation	

[a]Included in the Initiative are 75 hES cell lines derived in 17 laboratories from these Forum members.

There are strong indications that some hES cell lines undergo spontaneous differentiation more readily than others under standardized conditions, and there is also anecdotal evidence that some lines differentiate more readily into particular lineages than do others. Large-scale comparison may provide the first firm data on the variability in differentiation among cell lines. The study is constructed so that several laboratories will be reporting data on the same cell lines, permitting some insight into how the properties of a line vary when handled in different laboratories.

Analysis of the correlation of the expression of various pluripotency markers will help select a set of surface antigens and genes that most reliably defines stem cell status, and may even yield clues into how the genes interact in stem cell maintenance at the molecular level. Existing data have not identified one gene or surface marker that universally defines stem cell status. But if a small and discrete set of markers and genes is found to decline consistently in a coordinate fashion during the early differentiation of all stem cell lines, this panel would comprise a phenotypic metric that provided a basis for comparing, for example, the effects of new culture methodology on stem cell maintenance in different laboratories. Quality control studies such as microbiological analysis and DNA fingerprinting will aid in the prevention of some trivial but all-too-common errors in mammalian cell culture, such as mycoplasma contamination or cross-contamination of cell lines. Finally, the study may help guide ethical review of efforts to derive new stem cell lines. If all 75 lines are roughly similar in stem cell phenotype, the argument for deriving additional cell lines using existing technologies cannot be strong, provided that the lines can be made routinely available worldwide.

Perhaps the most important outcome of this effort will be the establishment of a precedent and a formal mechanism for large-scale international collaboration in this area. The scientific community has been nearly unanimous in arguing the potential benefits of embryonic stem cell research to the public, and it has a collective responsibility to ensure that this potential is thoroughly explored in a timely fashion. The opportunities and challenges in the field are sufficiently complex, and the resources required to address them so substantial, that the field will need to rely not only on individual investigator project grants but also on large, international collaborations along the lines of the Human Genome project. Areas for future exploration might include the maintenance of genetic stability of hES cells; development and validation of new culture methodologies; development of other platform technologies such as genetic manipulation; safety issues; and preclinical models of cell-based therapies. Thus, the pilot phase of the International Stem Cell Initiative represents a first step toward a truly integrated international approach to the refinement and propagation of hES cell technology for research and regenerative medicine.

1. Thomson, J.A. *et al. Science* **282**, 1145–1147 (1998).
2. Keller, G. *Genes Dev.* **19**, 1129–1155 (2005).
3. Pera, M.F. & Trounson, A.O. *Development* **131**, 5515–5525 (2004).
4. Sperger, J.M. *et al. Proc. Natl. Acad. Sci. USA* **100**, 13350–13355 (2003).
5. Richards, M., Tan, S.P., Tan, J.H., Chan, W.K. & Bongso, A. *Stem Cells* **22**, 51–64 (2004).
6. Dvash, T. *et al. Hum. Reprod.* **19**, 2875–2883 (2004).
7. Brimble, S.N. *et al. Stem Cells Dev.* **13**, 585–597 (2004).
8. Bhattacharya, B. *et al. Blood* **103**, 2956–2964 (2004).
9. Abeyta, M.J. *et al. Hum. Mol. Genet.* **13**, 601–608 (2004).
10. Rao, R.R. *et al. Biotechnol. Bioeng.* **88**, 273–286 (2004).

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