Unnatural selection of cultured human ES cells?

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Human embryonic stem (ES) cells maintained in culture can develop chromosomal abnormalities.

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Human ES cells, unlike almost all cells derived from adult or fetal human tissues, have the potential to provide an indefinitely renewable source of a wide range of normal cell types for use in research and regenerative medicine. The many revolutionary applications envisioned for human ES cells are based not only on their demonstrated properties of immortality and pluripotentiality, but also on the evidence to date that they maintain a normal genetic makeup during extensive propagation, expansion and manipulation *in vitro*. The report of Draper *et al.*[1] in this issue raises a significant warning concerning the genetic stability of these cells. Several widely used ES cell lines—representing a quarter of those available for use in research funded by the US National Institutes of Health (Bethesda, MD, USA)—repeatedly developed specific karyotypic abnormalities *in vitro* when grown under established culture regimens by experienced workers (**Fig. 1**).

Though the subject has not been systematically studied, it is known that mouse ES cells can develop karyotypic abnormalities during passage in culture, and that these abnormalities are, not surprisingly, associated with a decreased capacity of the cells for germ line colonization in chimeras obtained after blastocyst injection[2,3]. Published results on the derivation of human ES cells have been reassuring. It is actually surprising, given the high rates of aneuploidy observed in human embryos cultured *in vitro*[4], that no cell lines with abnormal karyotypes have been reported, and indeed, most studies have indicated that euploidy, at the level of resolution of a G-banded karyotype, is maintained for at least 30–40 passages *in vitro*[5–9].

Now, Draper *et al.* have shown that specific abnormalities resulting in overrepresentation of chromosomes 17q and 12 appeared on multiple occasions in cultures of four separate ES cell lines; the changes emerged in most cases after extended passage (beyond passage 30 from derivation). Overrepresentation of these regions of the genome is also characteristic of the spontaneous development of germ cell tumors of the testis in man. The stem cells of these tumors are malignant counterparts of pluripotent stem cells, and it is possible that chromosomes 17q and 12 contain genes whose overexpression confers some survival or proliferative advantage to pluripotent stem cells.

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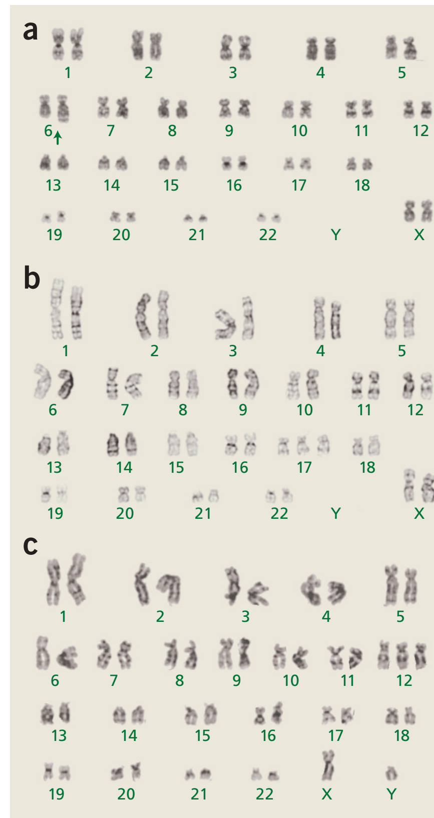

Figure 1 Karyotypic abnormalities in cultured human ES cells resulting in trisomy for 17q (**a**), 17 (**b**) and 12 (**c**) (ref. 1).

The Draper *et al.* study does not enable us to draw strong conclusions about how general their findings may be. First, most of the data were obtained on only two lines of human ES cells. Second, the specific karyotypic abnormalities that predominated in the cultures differed between the two laboratories involved in the study. Finally, because the data do not allow us to make even a coarse estimate of the frequency per cell generation at which the abnormalities occurred, it is hard to assess how common the problem is, or to compare these results with other studies, or even to make comparisons between the two laboratories involved in this work.

It is important not to draw the conclusion that these results reflect a high intrinsic level of genetic instability of human ES cells grown *in vitro*. Apart from the caveats regarding the generality of the findings, it may be that the results stem from particular aspects of the cell culture methods or the period for which the cells were cultured under these conditions. Although both laboratories participating in the study used culture media incorporating a proprietary serum replacement and fibroblast growth factor 2, there were differences; in one laboratory, the cells were grown without a feeder layer and were subjected to clonal derivation, whereas in the other laboratory, a feeder cell layer was employed and cells were passaged at relatively higher density. Most, though not all, reports of human ES cell derivation have used feeder cell layers and serum-containing medium, and have not routinely attempted low-density passage of the cells.

Further systematic, interlaboratory comparison of the genetic stability of different ES cell isolates, grown under several carefully defined conditions at defined passage levels, will be required to identify both the scope of the problem of karyotypic instability and the culture conditions, if any, that predispose to it. If a culture system is in any way suboptimal, it presents a selective barrier to long-term maintenance of cells *in vitro*; genetic variants arising spontaneously with a growth or survival advantage may overcome this barrier and come to dominate the cell population. It is not yet known whether the cytogenetic changes observed in this study actually do confer a selective advantage on ES cells *in vitro*, but further study of this issue might yield important clues about genes involved in the maintenance of pluripotent cells *in vitro*.

The present report highlights some other very important issues. In this study, and other published descriptions of human ES cells, cell lines were examined for genetic lesions using chromosome banding or, less frequently, fluorescence *in situ* hybridization. Microdeletions, or minor rearrangements or amplifications, would escape detection by these

techniques, as would point mutations in genes regulating growth, survival or differentiation. It will be important to apply higher resolution methodology, such as comparative genomic hybridization, to assess the genetic health of human ES cells during long-term culture, particularly as novel culture methods are developed and introduced into practice.

Another important lesson is that the karyotypically abnormal cell lines in this study still expressed canonical ES cell markers and retained the ability to differentiate *in vitro*, as indeed do certain cell lines derived from human embryonal carcinoma. Unlike the case of mouse cells, to which the bright-line criterion of germline chimerism can be applied, with human cells we lack robust biological markers that allow us to distinguish between normal ES cells and ES cells that have undergone minor genetic changes that might confer growth or survival advantages, or reduce their capacity for differentiation. Identification of such markers will be an important aspect of the evolution of standards for human ES cells.

Finally, no study to date has assessed the epigenetic stability of human ES cells. Changes in methylation status or in chromatin structure during culture *in vitro* may strongly affect the cells' capacity for differentiation. Though these issues may seem daunting, it is reassuring that mouse ES cells have been used throughout the world for over 20 years to produce normal live chimeric offspring, in the absence of any detailed or systematic assessment of the occurrence of spontaneous genetic or epigenetic alterations and their impact on developmental potential.

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Protein interaction maps on the fly

Peter Uetz & Michael J Pankratz

A protein interaction map for the fruit fly *Drosophila melanogaster* promises to facilitate functional analysis of many eukaryotic proteins.

Unraveling the ways in which proteins interact with each other on a genome-wide level is one of the main goals of proteomics research. Until now, large-scale protein interaction maps have been published only for yeast[1–3] and bacteria[4]. Now, in a paper published in *Science*, Giot *et al.*[5] report the largest protein interaction map to date and the first genome-wide study for a multicellular organism, the fly. Key to this work was the development of computational and statistical methodologies to extract relevant protein-protein interactions, which are presented in this issue[6].

Building the interaction map required a *tour de force* of PCR to amplify all 14,000 predicted *D. melanogaster* open reading frames (ORFs), of which more than 12,000 worked successfully. These PCR products were then cloned into two-hybrid bait (protein of interest) and prey (interactors) vectors, yielding roughly 11,000 clones each. This achievement is amazing by itself, given that sequencing of the PCR products represents a serious expressed sequence tag project on its own, which may allow biologists to identify many new exons and introns if investigated further. Next, all of the clones were screened against each other using methods Giot *et al.* have employed for the yeast genome. The fly project, however, proceeded on a vastly greater scale, requiring isolation of 45,417 two-hybrid positive colonies, from which 35,151 prey clones were obtained and sequenced. This approach generated 10,021 protein interactions involving 4,500 proteins.

Giot *et al.* then went one step further using their whole bait collection to screen a two-hybrid cDNA library prepared from embryonic, larval and adult tissues. Again, these screens resulted in another 45,962 positives of which 31,760 clones were successfully sequenced. This more traditional approach yielded 10,782 interactions involving 5,200 proteins. Thus, altogether Giot *et al.* churned out a whopping 20,405 interactions, dwarfing all previous efforts by at least four- to fivefold.

Nevertheless, two-hybrid screens are infamous for their false-positive rate; thus, one wonders about the reliability of these data. To address this issue, Giot *et al.* have developed fairly sophisticated statistics for the analysis of their protein network and Bader *et al.*[6] elaborate on this work, using yeast data as a model. In brief, both papers exploit topological and other information in a network to calculate confidence scores for each protein interaction. For example, two-hybrid interactions are considered low-confidence interactions if the interacting proteins are not found in a protein complex. Similarly, if proteins that have been found in a complex are far apart from each other in a two-hybrid map, the confidence score for this interaction decreases. Giot *et al.* use many more such parameters, including the reproducibility of interactions, the frequency with which bait and prey show up in a screen (indicating sticky and unspecific interactions) and evolutionary conservation of an interaction when compared with the yeast data set. After optimizing their statistical model, Bader *et al.* validated it by correlating their calculated confidence scores to independently collected information, such as gene ontology annotation (functional classification and subcellular localization) and gene expression data. As a result of such analyses, Giot *et al.* estimated that the fraction of 'biologically relevant' interactions may be as low as 11% in their whole data set, but around 38% in their high-confidence subset of 4,679 proteins and 4,780 interactions.

Given these quality-controlled protein interactions, what have we gained from this plethora of information? First, extrapolating from our experience with yeast, it is clear that although Giot *et al.* identified a huge number of interactions, they may represent only a fraction of all protein interactions in flies, which may harbor 50,000 or more. In addition, the number of essential interactions remains unresolved.

Second, the fly network has generated many novel hypotheses for biologists working on individual proteins, pathways or processes to investigate (see **Fig. 1**).

Third, 44% of all fly genes have homologs in the human genome, making *D. melanogaster* an excellent model system for studying normal and pathological processes; therefore, the protein interaction map should provide new avenues of investigation for medical research. In fact, Giot *et al.* explicitly highlight

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