STEM CELLS

Cultural shifts

A study of the genetic variation in 17 human embryonic stem cell lines shows hundreds of changes, some associated with cancer.

When grown in culture, human embryonic stem cells accrue mutations. Many of these changes are similar to those observed in cancer cells and may affect the cells' suitability for use in therapies or even for studying disease. However, detecting such changes has been difficult, as has been characterizing their accumulation in different cell lines over time. A group of scientists from seven countries recently reported the most comprehensive characterization so far of genomic stability in human embryonic stem cell (hESC) lines. Led by Elisa Närvä and Riitta Lahesmaa of the Turku Centre of Biotechnology in Finland, the scientists analyzed 17 hESC lines maintained in different laboratories and compared variation to that found in genomes from the HapMap project, which provides a reference adult population.

Previous studies of genetic stability in hESCs have probed fewer lines for either limited numbers of differences or for larger differences that can be made apparent on intact chromosomes. This analysis cataloged variation more extensively, focusing on two classes of genetic differences: single-nucleotide polymorphisms and copy-number variations (CNVs), which are genomic regions that are amplified or deleted. To do so, the researchers used a hybridization array containing nearly a million probes for single-nucleotide polymorphisms, plus nearly another million probes to detect CNVs.

These analyses identified 843 areas with CNVs ranging in size from 50 to 3,000 kilobases. The number and sizes of these CNVs were similar to those identified in the genomes of 90 Caucasian individuals sampled from the HapMap project. However, the distribution of these CNVs on chromosomes did differ. And although HapMap CNVs represented gains and losses of genomic regions equally, nearly three-quarters of CNVs in hESCs were gains or amplifications.

Follow-up studies indicated that CNVs often affected gene expression and that the biological function of many of these genes was associated with cancer. Additionally, the researchers' analysis showed that one of the cell lines (FES21) lost heterozygosity in the short arm of chromosome 16, though standard karyotyping of this hESC line had not previously detected this change; loss of heterozygosity, in which either the maternal or paternal region of a chromosome is deleted and its counterpart is duplicated, is frequently associated with cancer cells.

Other studies have indicated that mutations that allow hESCs to thrive in culture promote cancer-like qualities. Accordingly the researchers analyzed genomes from several samples of the same line that had been maintained in culture for many cell divisions (more than 50 passages) and compared them to those with fewer cell divisions (fewer than 50 passages), searching for regions affected by time in culture. The data indicated that new loss-of-heterozygosity sites arose at an average rate of 1.3 per passage. These had an average size of 1,000 kilobases and occurred on nearly every chromosome.

The researchers also asked whether the variations detected in hESCs occurred in different lines. They identified seven amplified and two deleted regions in eight or more samples. One of these, a deletion of tumor suppressor *HIC2*, they found in 28% of hESC samples but in fewer than 5% of HapMap samples. This change is likely to be culture-induced because the reseachers observed it in genomes of later-passage samples but not in earlier ones of the same lines.

Although these results do not indicate that stem cells cannot be used therapeutically, they do indicate that time in culture should be limited for cells intended for therapeutic use. Even basic researchers need to be careful: "Small changes acquired during culture may have profound influence on the phenotype of molecular mechanisms that are under investigation," says Lahesmaa. In addition to monitoring and analysis, she says, results from experiments should be verified on multiple cell lines.

Besides looking into the functional importance of mutations acquired during cell culture, Lahesmaa and colleagues plan to examine epigenetic changes and to conduct similar genomic studies of the genetic variations that occur when somatic cells are reprogrammed into pluripotent stem cells.

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RESEARCH PAPERS

Närvä, E. *et al.* High-resolution DNA analysis of human embryonic stem cell lines reveals culture-induced copy number changes and loss of heterozygosity. *Nat. Biotechnol.* **28**, 371–377 (2010).