

their GH pulses are much smaller when studied *in vitro*?

Using GH-GFP transgenic mice and custom-made computer software, these investigators were able to identify and localize the 3-D position of the labeled somatotrophs within the pituitary gland. Examination of fixed pituitaries from adult male mice revealed a connected 3-D, multi-cellular system comprised of numerous intercrossing strands of single GH cells with larger cell clusters at the intersections. This GH multi-cellular assembly withstood dispersion by a high-pressure *in vivo* perfusion procedure, and was shown to be linked by focal adherens junctions containing β -catenin.

The system was shown to be both functional and plastic. Comparing the volume-to-surface ratios of the GH cell clusters within the lateral and median pituitary zones, the ratios were similar in prepubertal animals. However, GH cell clusters increased in the lateral zones from puberty to adulthood, and then returned to prepubertal geometries in the oldest mice. Cell clustering was prevented by prepubertal castration of male mice, without a significant change in GH cell density in the lateral zones; organizational geometry was the important factor for the pubertal increase in growth. Multi-cellular calcium recordings of GH-EGFP cells in acute pituitary slices were measured as a marker of cell-cell connectivity in hormone release. No large-scale cell connectivity was observed during spontaneous electrical activity. This increased in the lateral pituitary zones following GH-releasing hormone (GHRH) stimulation, leading to temporally precise, synchronized, recurrent calcium spikes that correlated with the frequency of small GH pulses reported in other studies; enzymatic dispersion of the GH cells prevented GHRH-stimulated calcium spike synchronization. GHRH also increased calcium spiking in the median pituitary zone by changing the cell connectivity into small islets of more highly functionally connected GH cells at some points in the system interspersed with functionally less connected GH cells.

The authors concluded that, "GH cells function as a geometry-driven network of cells, connected to each other by adherens junctions." It logically follows that disruption

of network architecture constitutes a novel mechanism for impaired GH release in pathological conditions, an issue the authors are pursuing in follow-up experiments.

Bonnefont X, Lacampagne A, Sanchez-Hormigo A, et al. Revealing the large-scale network organization of growth hormone-secreting cells. *Proc Natl Acad Sci.* 2005;102:16880–16885.

Editor's Comment: *A 3-D approach to functional analysis of the GH cell network provided novel and interesting insights into its physiology that were heretofore unobtainable. Because it is noninvasive and provides sensitive, real-time data of cellular and molecular events within their biological context,¹ in vivo bioluminescent imaging has recently emerged as a powerful new approach to elucidate physiologic and pathophysiologic mechanisms. It can be used grossly, such as monitoring rejection of transplanted tissues^{2,3} or growth of cancer metastases.⁴ It can also be used to study protein-protein interactions,⁵ transcription,⁶ and gene silencing.⁷ Bioluminescent or fluorescent imaging holds great promise as a means of drug testing, both for therapeutic efficacy⁸ and potential effects on normal tissues,⁹ as well as in vivo evaluation of gene therapy strategies.¹⁰*

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Genomic Alterations in Human Embryonic Stem Cells

The potential use of human embryonic stem cells (hESC) is an exciting but controversial area in medicine today. In concept, hESC cells might be able to repair and/or regenerate damaged tissues and replace injured cells. It is often assumed that after harvesting, these cells are genetically stable, even though they must be expanded substantially through repeated cell division to generate enough cells for current experiments and for possible future therapeutic uses. However, like all dividing cells, it is probable that cultured hESC undergo a low level of spontaneous mutation, which in some cases could adversely affect their therapeutic potential. Maitra et al examined this issue by comparing several parameters

of genomic stability in 9 hESC lines that were available as both early and late passage cells, ie, early and late passage paired cell lines. Cells normally stop dividing when they reach high density in culture, but they will start dividing again if diluted. Passage refers to this dilution process; it is a crude measure of the number of times cells have divided, ie, late passage cells have divided many more times than early passage cells.

The authors used 3 assays to search for alterations of cellular DNA: nuclear DNA copy number, mitochondrial DNA sequence, and gene promoter methylation. In the first case, initial Affymetrix high-density array analysis of approximately 115 000 single nucleotide polymorphisms

(SNPs) distributed across the genome showed no significant differences between the early and late passage hESC. However, further analysis revealed copy number alterations in late but not early passage cells from 4 of the 9 paired cell lines. The alterations ranged from large genomic regions of amplification or deletions, such as amplification of the entire chromosome 17q arm, to discrete changes such as a 2-Mb amplification that included the MYC oncogene. These changes were verified by *in situ* hybridization (FISH) or quantitative genomic PCR.

Next, they screened the mitochondrial genome, which is often mutated in cancer, again using array technology. Sequence alterations were detected in 2 of the late passage hESC cell lines that were not observed in early passage cells from the same cell lines.

Promoter methylation, an epigenetic phenomenon observed in almost all cancers, was assessed in a panel of 14 genes known to be differentially methylated in cancer cells. Differential methylation of 3 genes was detected in late passage cells. For one gene, RASSF1 – a putative tumor suppressor gene, increased methylation was found in late but not early passage cells from 7 of the 9 paired hESC lines.

In conclusion, the authors suggest that most but not all

hESC lines maintained in cell culture acquire clonal DNA alterations over time. Many of these alterations are similar to what has been observed in cancer, such as loss of tumor suppressor genes or amplification of oncogenes. These alterations may provide a growth advantage that allows the cells that harbor them to dominate late passages. The authors acknowledge that much more work is needed to better define the nature of these alterations and their functional consequences. However, they argue that their findings underscore the need to periodically monitor hESC lines before they are used in *in vivo* applications and that some late-passage hESC may be unusable for therapeutic purposes due to genomic alterations over time.

Maitra A, Arking DE, Shivapurkar N, et al. Genomic alterations in cultured human embryonic stem cells. *Nat Genet.* 37:1099–1103.

Editor's Comment: *It is clear that hESC have great potential in regenerative medicine. However, this paper illustrates that the field is still relatively young with many troublesome issues, such as long-term genomic fidelity, must be resolved before it can be applied clinically.*

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Growth, Genetics & Hormones is supported by an unrestricted educational grant from **Genentech, Inc.**

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