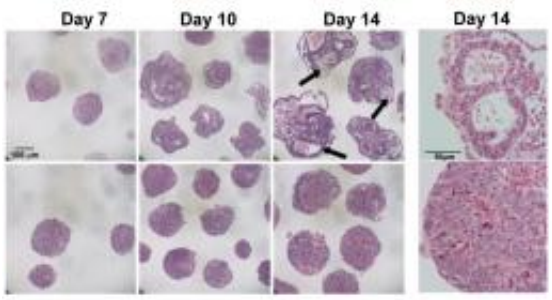
Genetic packing: Successful stem cell differentiation requires DNA compaction, study finds

May 11 2012, by Abby Robinson



Hematoxylin and eosin (H&E) staining of sections of wild-type (top row) and H1 triple-knockout (bottom row) embryoid bodies. After 14 days in rotary suspension culture, the wild-type embryoid bodies showed more differentiated morphologies with cysts forming (black arrows) and the knockout embryoid bodies failed to form cavities (far right). (Credit: Yuhong Fan)

(Phys.org) -- New research findings show that embryonic stem cells unable to fully compact the DNA inside them cannot complete their primary task: differentiation into specific cell types that give rise to the various types of tissues and structures in the body.

Researchers from the Georgia Institute of Technology and Emory University found that chromatin compaction is required for proper embryonic stem cell differentiation to occur. Chromatin, which is composed of histone proteins and DNA, packages DNA into a smaller

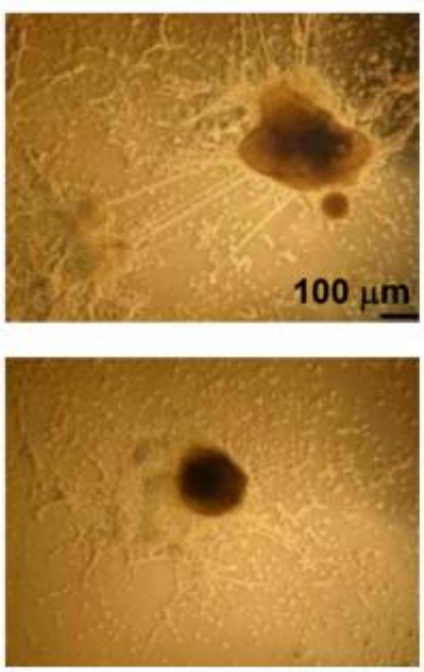
volume so that it fits inside a cell.

A study published on May 10, 2012 in the journal PLoS Genetics found that embryonic stem cells lacking several histone H1 subtypes and exhibiting reduced chromatin compaction suffered from impaired differentiation under multiple scenarios and demonstrated inefficiency in silencing genes that must be suppressed to induce differentiation.

“While researchers have observed that embryonic stem cells exhibit a relaxed, open chromatin structure and differentiated cells exhibit a compact chromatin structure, our study is the first to show that this compaction is not a mere consequence of the differentiation process but is instead a necessity for differentiation to proceed normally,” said Yuhong Fan, an assistant professor in the Georgia Tech School of Biology.

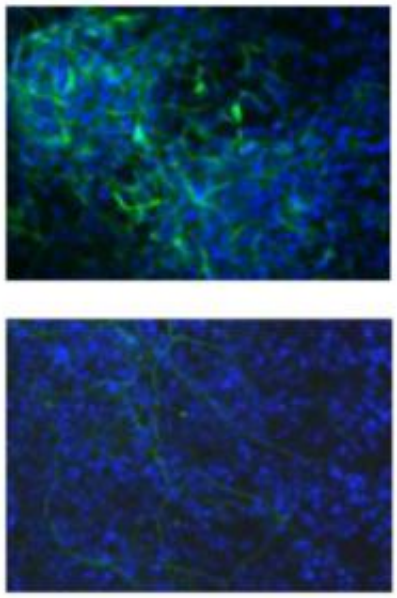
Fan and Todd McDevitt, an associate professor in the Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University, led the study with assistance from Georgia Tech graduate students Yunzhe Zhang and Kaixiang Cao, research technician Marissa Cooke, and postdoctoral fellow Shiraj Panjwani.

The work was supported by the National Institutes of Health’s National Institute of General Medical Sciences (NIGMS), the National Science Foundation, a Georgia Cancer Coalition Distinguished Scholar Award, and a Johnson & Johnson/Georgia Tech Healthcare Innovation Award.



Phase contrast images showing that H1 triple-knockout (bottom) embryonic stem cells were unable to adequately form neurites and neural networks compared to wild-type embryonic stem cells (top). (Click image for high-resolution version. Credit: Yuhong Fan)

To investigate the impact of linker histones and chromatin folding on stem cell differentiation, the researchers used embryonic stem cells that lacked three subtypes of linker histone H1 — H1c, H1d and H1e — which is the structural protein that facilitates the folding of chromatin into a higher-order structure. They found that the expression levels of these H1 subtypes increased during embryonic stem cell differentiation, and embryonic stem cells lacking these H1s resisted spontaneous differentiation for a prolonged time, showed impairment during embryoid body differentiation and were unsuccessful in forming a high-quality network of neural cells.



Immunostaining of wild-type (top) and H1 triple-knockout (bottom) cultures under a neural differentiation protocol. The H1 triple-knockout cells were defective in forming neuronal and glial cells and a neural network, which is essential for nervous system development. (Credit: Yuhong Fan)

“This study has uncovered a new, regulatory function for histone H1, a protein known mostly for its role as a structural component of chromosomes,” said Anthony Carter, who oversees epigenetics grants at NIGMS. “By showing that H1 plays a part in controlling genes that direct embryonic stem cell differentiation, the study expands our understanding of H1’s function and offers valuable new insights into the cellular processes that induce stem cells to change into specific cell types.”

During spontaneous differentiation, the majority of the H1 triple-knockout embryonic stem cells studied by the researchers retained a tightly packed colony structure typical of undifferentiated cells and expressed high levels of Oct4 for a prolonged time. Oct4 is a pluripotency gene that maintains an embryonic stem cell’s ability to self-

renew and must be suppressed to induce differentiation.

“H1 depletion impaired the suppression of the Oct4 and Nanog pluripotency genes, suggesting a novel mechanistic link by which H1 and chromatin compaction may mediate pluripotent stem cell differentiation by contributing to the epigenetic silencing of pluripotency genes,” explained Fan. “While a significant reduction in H1 levels does not interfere with embryonic stem cell self-renewal, it appears to impair differentiation.”

The researchers also used a rotary suspension culture method developed by McDevitt to produce with high efficiency homogonous 3D clumps of embryonic stem cells called embryoid bodies. Embryoid bodies typically contain cell types from all three germ layers — the ectoderm, mesoderm and endoderm — that give rise to the various types of tissues and structures in the body. However, the majority of the H1 triple-knockout embryoid bodies formed in rotary suspension culture lacked differentiated structures and displayed gene expression signatures characteristic of undifferentiated stem cells.

“H1 triple-knockout embryoid bodies displayed a reduced level of activation of many developmental genes and markers in rotary culture, suggesting that differentiation to all three germ layers was affected.” noted McDevitt.

The embryoid bodies also lacked the epigentic changes at the pluripotency genes necessary for differentiation, according to Fan.

“When we added one of the deleted H1 subtypes to the embryoid bodies, Oct4 was suppressed normally and embryoid body differentiation continued,” explained Fan. “The epigenetic regulation of Oct4 expression by H1 was also evident in mouse embryos.”

In another experiment, the researchers provided an environment that would encourage embryonic stem cells to differentiate into neural cells. However, the H1 triple-knockout cells were defective in forming neuronal and glial cells and a neural network, which is essential for nervous system development. Only 10 percent of the H1 triple-knockout embryoid bodies formed neurites and they produced on average eight neurites each. In contrast, half of the normal embryoid bodies produced, on average, 18 neurites.

In future work, the researchers plan to investigate whether controlling H1 histone levels can be used to influence the reprogramming of adult cells to obtain induced pluripotent stem cells, which are capable of differentiating into tissues in a way similar to embryonic stem cells.

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