Difference between induced pluripotent and embryonic stem cells highlighted in a disease model

Researchers from the Children's Hopsital Boston, MA, USA, have highlighted differences in the ability of induced pluripotent stem (iPS) cells to model a human genetic disease, when compared with the behavior of embryonic stem cells (ESCs) in the same disease model.

The researchers investigated iPS cells made from the skin cells of three patients with fragile X syndrome, an inherited genetic syndrome causing mental retardation in boys. Whilst nearly all the genes within the cells were fully reversed back to a stem cell-like state, the disease-causing gene was not affected. "This is the first example where we can clearly show iPS cells and ESCs behave differently in a disease model," said co-senior author George Daley, Director of the Stem Cell Transplantation Program at the medical center.

Fragile X syndrome causes behavioral symptoms that overlap with autism. The mutation that silences the fragile X gene, *FMR1*, results in a lack of fragile X protein. "It's known that the fragile X protein regulates the expression of receptors at the synapse between nerve cells," Daley explained. "In the absence of the protein, nerve cells express too much of an excitatory receptor." Sometimes, the mutation alone is not enough to cause disease. "In rare cases, people can have the full mutation, but the gene is still expressed," said co-first author Achia Urbach, a postdoctoral fellow in the Daley laboratory and a former graduate student in the Benvenisty laboratory in Jerusalem, where an ESC model of fragile X syndrome was established 3 years ago.

In the last 3 years, the technique for reprogramming adult cells has been developed with the aim of studying human disease and developing new therapies; Daley's team themselves have developed more than a dozen iPS cell lines for a range of diseases. Now, this new research has thrown up new considerations for researchers in the field. "This raises a general caution for using iPS as a faithful reflection of a disease process," Daley advised. "There are lots of conditions where you have gene defects that lead to gene silencing. Such conditions may not be faithfully modeled by iPS cells. Fragile X is a disease where using ESCs as a tool is essential."

However, while differences certainly exist between the iPS and embryonic fragile X stem cells, the importance of those differences is still up for debate. Some differences may be useful for different type of studies. "On one hand, iPS cells are not as good for modeling the inactivation of the gene, but on the other hand, they may be a better model for studying neurons lacking expression of the gene," suggested the co-senior author Nissim Benvenisty, who is the director of the Stem Cell Unit at the Hebrew University of Jerusalem. "New insights into fragile X have stimulated clinical trials of drugs that block the overactive excitatory receptors in nerve cells. Early results hint that these drugs might ameliorate the condition of fragile X," concluded Daley. "With our stem cell models – diseases in a dish, if you will – we can test whether the drugs will reverse abnormal connections at the synapses that we think are at the basis of this condition."

Sources: Children's Hospital Boston, USA: www.childrenshospital.org/newsroom/Site1339/mainpageS1339P1sublevel625.html; Urbach A, Bar-Nur O, Daley GQ, Benvenisty N: Differential modeling of Fragile X syndrome by human embryonic stem cells and induced-pluripotent stem cells. Cell Stem Cell *6(5), 407–411 (2010).*

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Gene silencing observed in iPS cells

Genes important for fetal development found to be inactivated within some iPS cell lines.

Recent research has led to the discovery that an important cluster of genes is inactivated in induced pluripotent stem (iPS) cells that do not have the full development potential of embryonic stem cells (ESCs). A group led by Konrad Hochedlinger at the Massachusetts General Hospital Center for Regenerative Medicine and the Harvard Stem Cell Institute have recently reported findings highlighting certain limitations that are still present within iPS cells.

Whilst iPS cells appear highly similar to ESCs, molecular differences between the two cell types have previously been observed, including within the control of gene expression and in the generation of live animals from the cells. In comparing the two cell types to obtain these data, many previous reports have compared cells obtained from unrelated animals. Hochedlinger's research team developed two genetically matched cell lines; after producing mice from ESCs, the team then prepared iPS cells from somatic cells harvested from the mice. In comparing the two cell lines, whilst live mice were successfully produced from two ESC lines, no animals were generated from the iPS cells. When the research team closely compared the RNA transcription profiles of the matched cell lines, significantly reduced transcription of two genes within the iPS cells was discovered. "We found that a segment of chromosome 12 containing genes important for fetal development was abnormally shut off in most iPS cells," Hochedlinger explains.

The researchers proceeded to examine more than 60 iPS cell lines developed from several types of cell, and a similar result was found in the majority of the cell lines analyzed, ultimately resulting in limited developmental potential of the gene silenced-iPS cell line. Importantly, however, the gene cluster was activated in a few of the iPS cell lines and in a landmark result, the researchers were able to successfully produce live animals from these iPS cells. "The activation status of this imprinted cluster allowed us to prospectively identify iPS cells that have the full developmental potential of embryonic stem cells," said Matthias Stadtfeld, a co-lead author of the report. "Identifying pluripotent cells of the highest quality is crucial to the development of therapeutic applications, so we can ensure that any transplanted cells function as well as normal cells. It's going to be important to see whether iPS cells derived from human patients have similar differences in gene expression and if they can be as good as embryonic stem cells – which continue to be the gold standard – in giving rise to the 220 functional cell types in the human body."

Nonetheless, the implications of these particular findings are significant within the iPS field, particularly with respect to characterization and derivation of the cell lines. As Hochedlinger warns, "These findings indicate we need to keep improving the way we produce iPS cells and suggest the need for new reprogramming strategies."

Sources: Massachusetts General Hospital, USA: www.mgh.harvard.edu/about/pressrelease.aspx?id=1226; Stadtfeld M, Apostolou E, Akutsu H et al.*: Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells.* Nature *465(7295), 175–181 (2010).*

Enhanced pluripotency achieved through gene insertion

Established human induced pluripotent stem (iPS) cells and human embryonic stem cells (ESCs) have been converted to a state of greater pluripotency than has previously been achieved by researchers led by Rudolf Jaenisch at the Whitehead Institute for Biomedical Research, MA, USA.

Mouse ESC research has provided a viable basis for studying ESCs, whilst avoiding any ethical and legal issues associated with human ESC research. However, differences between cells from the two species have led to concerns regarding the reproducibility of mouse ESC methods within human cells. Specifically, human ESCs and iPS cells depend on different cell signaling pathways and express different sets of genes, resulting in greater complications involved in work with them. The human cells can be referred to as 'primed' for differentiation, compared with the mouse cells, which are 'naive' in their status. Jacob Hanna, a postdoctoral researcher in the Jaenisch laboratory, inserted two genes used to create iPS cells into established human ESC and iPS cell lines, and discovered that the cells became morphologically and biochemcially more similar to their mouse counterparts after approximately 3 weeks. "This is a previously unknown pluripotent state in human cells," said Hanna "It's the first time these cell types have approached the flexibility found in mouse ESCs."

Hanna then went on to screen hundreds of small molecules for candidates that might mimic the function of the genes, eventually discovering a cocktail of four molecules that converts established human ESCs and iPS cells to the 'naive' state characteristic of mouse ESCs. "I think this really opens things up, and gives us the possibility to define the biological properties of these new cells," Jaenisch described. "For example, we can study whether gene targeting, which is highly efficient in mouse ESCs but exceedingly inefficient in traditional human ESCs, is improved in the new naive human ESCs."

Sources: Whitehead Institute for Biomedical Research, MA, USA: www.wi.mit.edu/news/archives/2010/rj_0503.html; Hanna J, Cheng AW, Saha K et al.*: Human embryonic stem cells with biological and epigenetic characteristics similar to those of mouse ESCs.* Proc. Natl Acad. Sci. USA *107(20), 9222–9227 (2010).*

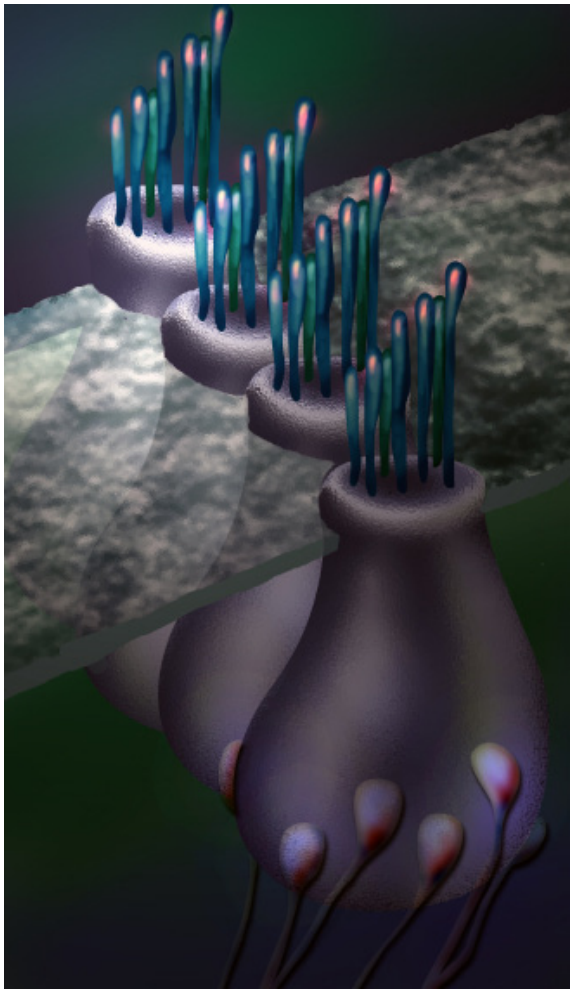
Functional mouse inner ear cells formed from induced pluripotent stem cells

Movement towards the development of better treatments for deafness achieved through formation of specialized hair cells from stem cells.

Scientists have developed a way to produce mouse cells that look and act just like the animal's inner-ear hair cells. The report, published by researchers at the Stanford School of Medicine, CA, USA, may prove to be key to helping scientists understand the molecular basis of hearing, in order to develop better treatments for deafness.

Stefan Heller, Professor of Otolaryngology at the university, led the research team that has developed the protocol. The researchers transformed **induced pluripotent** stem cells formed from reprogrammed mouse fibroblasts, as well as embryonic stem cells, into the sensory inner-ear cells. "We knew it was really working when we saw them in the electron microscope," Heller enthused. "They really looked like they were more or less taken out of the ear."

Hair cells are present deep inside the ear and translate vibrations detected in the air into sound. Hearing loss occurs when a significant number of these cells are lost or damaged and the *in situ* regeneration of these cells within humans is not possible. "One of the roadblocks to understanding the molecular basis of hearing is the paucity of hair cells available for study," Heller explains. "While researchers will ultimately need human hair cells, the mouse version is a good model for the initial phases of experimentation."



The successful production of these specialized cells from induced pluripotent stem cells is a major step towards possible stem cell transplantation therapies. "Our study offers a protocol to generate millions of functional hair cells from a renewable source," commented Heller, who came up with the idea of creating the hair cells from stem cells 10 years ago. "We can now generate these cells and don't have to go through dozens of mice for a single experiment. This allows us to do molecular studies with much higher efficiency."

Sources: Stanford School of Medicine, USA: http://med.stanford.edu/ism/2010/may/hair-cell.html; Oshima K, Shin K, Diensthuber M et al.*: Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells.* Cell *141(4), 704–716 (2010).*

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