Chromosome shutdown

By Tracey Baas, Senior Editor

A new technique to shut down chromosome 21 provides the clearest window yet into the cellular pathologies that underlie Down syndrome.[1] The findings will reshape how the condition is modeled and, in the longer term, could point to new disease targets or even a strategy to remove the extra chromosome.

Down syndrome occurs when an individual has three rather than two copies of chromosome 21, and it is the leading genetic cause of intellectual disabilities. Other Down syndrome health issues include congenital heart defects, early onset Alzheimer's disease (AD), premature aging and some forms of leukemia.

The cellular pathologies of trisomy 21 are likely to come from elevations in gene products, which could be tied to a protein-encoding gene directly or to a non–protein-encoding gene with a regulatory function.[2]

Efforts to correlate which genetic changes lead to which cellular pathology have been imprecise because researchers have had to compare disomic with trisomic cells from different humans. Thus, genetic polymorphisms and differences in patient age and medical history, as well as in cell culture conditions, have confounded such comparisons.[3–7]

Polymorphisms in the natural genetic background of the individual are speculated to have an important role in the variability of phenotypic severity seen in Down syndrome.

To eliminate individual variation due to genetic polymorphisms or make an isogenic comparison, a team from the **University of Massachusetts Medical School** and **Sangamo BioSciences Inc.** developed a technique to compare the same person's cells with and without trisomy 21.

In induced pluripotent stem (iPS) cells derived from a patient with Down syndrome, the researchers used Sangamo's zinc-finger nucleases to insert inducible *X inactive specific transcript* (non–protein-encoding) (*XIST*) into chromosome 21.

Normally, *XIST* produces a very large piece of noncoding RNA that shuts down one of the X chromosomes, triggering condensation of the chromatin to form an inactive Barr body.

In the iPS cells, induction of the newly inserted transgene resulted in expression of *XIST* noncoding RNA that coated chromosome 21 and triggered chromosome inactivation.

The researchers used fluorescence *in situ* hybridization (FISH), genome-wide expression profiling and methylomics to confirm the inactivation of chromosome 21.

iPS cells with induced *XIST* expression and chromosome 21 inactivation developed into neural rosettes, a signature of neural progenitor cells. In the cells that lacked induced *XIST* expression, however, neural rosette formation was delayed.

By using iPS cells from the same patient, the researchers were able to observe and compare the kinetics of neural differentiation associated with trisomy 21 without the confounding variability that occurs in comparisons of iPS cell lines from a trisomic individual and a disomic individual.

Results were published in *Nature*.

Breaking down trisomy 21

Next steps could include using the isogenic model to pinpoint specific genes or pathways that contribute to the underlying pathologies associated with Down syndrome, such as early AD or leukemia.

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> ***—Jeannie Lee, Harvard Medical School***

"Despite much progress in understanding Down syndrome, no human gene has yet been conclusively linked to causing a specific trisomy 21 phenotype," said André Mégarbané, professor of molecular biology and cytogenetics at **Saint Joseph University**.

He thinks the new system "could provide candidate genes associated with the syndrome that could be tested in mouse models for Down syndrome, evaluated at different stages of development and hopefully targeted with different therapeutic medicines."

Jeannie Lee, professor of genetics and pathology at **Harvard Medical School**, agreed the method could provide a useful system for understanding the pathology of Down syndrome. "The work is an exciting proof of concept that *XIST* can be used to inactivate chromosome 21 and balance chromosome 21 gene products in trisomic cells," she said. In 1996, Lee used transgenic analysis to show that coating of autosomes by *XIST* RNA resulted in silencing of that autosome. At the time, she was working in the laboratory of Rudolf Jaenisch, professor of biology at the **Massachusetts Institute of Technology**.

But David Russell noted that two active chromosomes 21 plus one inactive chromosome 21 might not necessarily represent disomy. "The *XIST* method shuts down the chromosome, but the chromosome is still there. Although many of the genes are probably shut down, some genes could still leak through to complicate comparison of the cells," he said. Russell is professor of medicine at the **University of Washington School of Medicine**.

Late last year, his team inserted *TKNEO*—a fusion gene encoding resistance to thymidine kinase and neomycin—into iPS cells derived from individuals with Down syndrome.[8] When selecting against *TKNEO*, spontaneous loss of chromosome 21 occurred through a natural cellular selection process, and iPS cells trisomic for chromosome 21 became disomic.

"Our system might be a little cleaner because the *TKNEO* method completely removes one chromosome 21," Russell told *SciBX*.

Regardless of approach, Russell said that differentiating iPS cells into somatic cell types complicates comparison between trisomic and disomic cells.

"We have differentiated iPS cells into neuronal and other cell types and have noticed it is very tricky to get consistent differentiation," he said. "There is a lot of clone to clone variation. So when comparing an iPS-derived trisomic neuronal cell to an iPS-derived disomic neuronal cell, you may be highlighting cell culture properties rather than pathology differences."

The upshot, he said, is that researchers need "to establish reproducible and robust phenotypes in order to make those comparisons."

The long road to chromosomal therapy

Although the general media jumped on the idea that chromosomal therapy could be used to shut down the extra chromosome in blood cells to prevent leukemia, the actual near-term applications of the *XIST* approach include using the model to discover genes and pathways relevant to the pathologies associated with Down syndrome that could be candidates for drug targeting.

"Developing *XIST*-corrected patient cells in a cell-based therapy is going to be a long, tough road," said Lee. "People will be interested in producing *XIST*-corrected neuronal cells, but brain tissue is an especially difficult one in which to attempt cell-based therapy."

Lee also said the production of *XIST*-corrected blood cells to protect individuals with Down syndrome from developing leukemia is likely to be unwieldy.

"Bone-marrow transplantation and chemotherapy are established procedures for treating leukemia and may still be the easier treatment," said Lee.

Mégarbané agreed. "Down syndrome patients have a tendency to develop pre-leukemia and are monitored closely. As soon as pre-leukemia is detected, treatments are started, which are very effective," he said.

"The real challenge of using *XIST* for chromosomal therapy is going to be delivery," said Mitchell Guttman, assistant professor of biology and biological engineering at the **California Institute of Technology**. "You may be able to deliver *XIST* into patient cells *ex vivo*, but delivering it *in vivo* will be a major challenge. First, you'll need to deliver *XIST* into cells of the embryo, and second, you will likely need to hit every cell to achieve success."

Guttman added, "Using it to treat a developmental disorder, like Down syndrome, would require targeting all cells during early development, but doing that is still quite impractical and currently hard to imagine."

Mégarbané was more sanguine and thinks some pathologies of Down syndrome could eventually be amenable to chromosomal therapy. He also said targeting all cells was not an absolute requirement. In some individuals with Down syndrome, not all cells have three copies of chromosome 21.

"It is hypothesized that the presence of cells with a normal number of chromosomes may result in a less severe presentation of Down syndrome [symptoms] such as impaired cognitive ability," Mégarbané said. "So even if only a percentage of cells could be corrected, that might be enough to benefit the patient."

One might want to attempt chromosome therapy during early embryonic development, for example, to prevent heart defects, which occur in about 50% of children with Down syndrome, he said.

Jeanne Lawrence, professor of cell and developmental biology and pediatrics at the University of Massachusetts Medical School and lead investigator of the *Nature* study, could not be reached for comment. The patent and licensing status of the work is unavailable.

Baas, T. *SciBX* 6(31); doi:10.1038/scibx.2013.815
Published online Aug. 15, 2013

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COMPANIES AND INSTITUTIONS MENTIONED

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