Scientists Establish New Haploid Human Embryonic Stem Cell Line

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NEW YORK (GenomeWeb) – In a groundbreaking study, scientists have reported the discovery of haploid human embryonic stem cells. The findings could change several assumptions about the biology of human development and have the potential to improve loss-of-function genetic screening.

As they reported today in *Nature*, the researchers — led by Ido Sagi and Nissim Benvenisty of Israel's The Hebrew University, and Dieter Egli of Columbia University — found that in particular lines of human embryonic stem cells (ESCs), approximately 1 in 100 will remain haploid. They were able to isolate these haploid ESCs and establish two cell lines carrying only one copy of each human chromosome.

Not only are the haploid ESCs viable and persistent, the scientists also found they could differentiate them into mature cells that remain haploid. "This is something that we did not expect," Benvenisty told GenomeWeb. "Most investigators, including us, would think we cannot get neurons or cardiomyocytes with only half the genome. But you can and that was a big, big surprise."

The study could provide the research community with new insights into basic biology, as well as ideas for improving genetic knockout screens, which would make the effects of loss-of-function mutations more readily observable.

While yeast has long been a lab mainstay because of its haploidy, the existence of mammalian haploid cells has only recently been demonstrated. In 2011, scientists from the University of Cambridge developed haploid mammalian stem cells from unfertilized mouse eggs. While these cells retained pluripotency, differentiating them into mature cell types always turned them diploid.

Benvenisty, who is the director of the Azrieli Center for Stem Cells and Genetic Research at The Hebrew University, said his lab's collaboration with Egli started several years ago, when Egli was able to coax unfertilized human eggs to divide and become embryonic stem cells.

Though the human embryonic stem cells seemed to turn diploid, Benvenisty said the team suspected there might be a minority of cells that remained haploid. "When eggs start to divide, they might start to divide with only one set [of chromosomes] and along the way they duplicate the genome. But maybe there are leftovers with only one set," he said.

Based on this hunch, Benvenisty had graduate student Tamar Lev-Golan count chromosomes in approximately 2,000 individual cells. Essentially, she karyotyped the equivalent of 100 amniocentesis samples. "Every time you do amniocentesis, you take single cells and count the chromosomes to see if they have a normal number," Benvenisty said. "Every time you do that,

you do it for twenty cells."

It was painstaking labor, but it paid off. Amongst all the diploid cells, Lev-Golan found two that were haploid. "That was a big celebration," Benvenisty said. Having established their presence, the lab began using a cell sorter to separate the haploid cells from the diploid cells.

The collaboration yielded two lines of haploid embryonic stem cells that divide indefinitely, although some will regularly become diploid. "Every month you have to go back to the cell sorter and purify the haploid cells," Benvenisty said. "It's a struggle but it's worth it."

One of the biggest surprises was how similar the haploid human ESCs are to diploid ones. There are clear differences, to be sure: haploid cells are smaller, exhibit less RNA, and the sex chromosomes function differently. But the global expression of genes was similar and in many respects they're the same. "So we thought if they look so normal, maybe we can convert them to any cell type that we like," Benvenisty said.

That led to the biggest, most shocking surprise. Not only did the haploid cells respond to the same differentiation cues as diploid cells, they remained haploid after reaching maturity. And the researchers were able to get this same result over and over, whether they tried turning the cells into neurons, cardiomyocytes, or pancreatic cells. "In a way, it is breaking the dogma that you cannot make mature cells with only half of the genome," Benvenisty said.

But the exact reasons that make this discovery possible are a little murky. "It's still puzzling," Benvenisty said. "There are reasons people, including us, thought you wouldn't be able to do it." One somewhat axiomatic hypothesis was that cells needed two copies of the genome to become mature and develop specific functions. Another was related to the balance of X chromosome expression. However, he added, "Clearly those reasons do not exist in our system."

In the paper, the authors wrote that haploid mammalian cells have already "proven invaluable for loss-of-function screens." Benvenisty's lab has generated a library of 10,000 haploid ESCs containing a single different mutation, induced via gene trap transposon system, and has used it in a proof-of-concept study to identify a specific pathway involved in metabolism. Since the gene trap system is more or less random, the lab is also developing a second library of haploid cells where the knockouts will be induced through CRISPR/Cas9 programmable gene editing.

"We are using this tool for cancer research and to understand development," Benvenisty said. "What was before useful in yeast, we can now do in human cells."

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