Breaking News: Growing Concerns Over STAP Cell Sources

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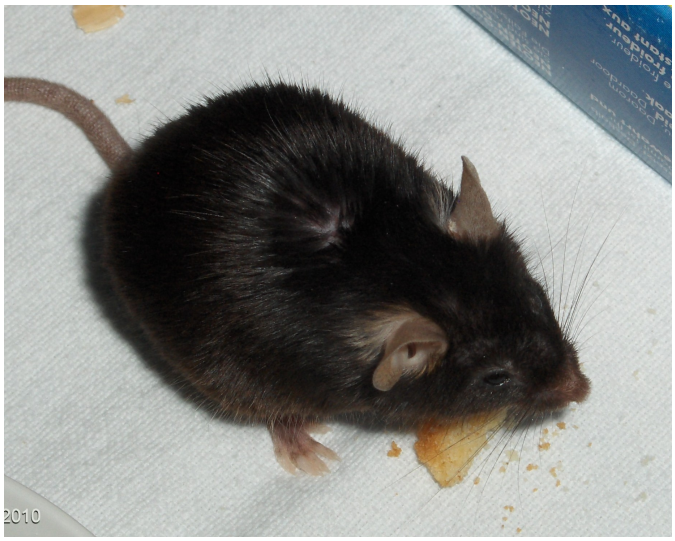

“Very sad results,” said cloning pioneer Teruhiko Wakayama about the latest troubling development in the STAP (“acid bath”) stem cell investigation.

“I simply feel sad,” echoed Shinichi Nishikawa, former deputy director of Riken Center for Developmental Biology.

Both scientists were speaking to *Bioscience Technology* about Wakayama’s recent discovery that two of several STAP stem cell batches made for two embattled *Nature* papers were apparently not derived from a 129 mouse strain, as Wakayama was told. They were derived from F1 and B6 mouse strains.

“This is just preliminary data, but strong data,” emailed Wakayama, an author on both *Nature* STAP papers. “Very sad results. However, that 129 mouse experiment data was not used for [primary conclusions of the] *Nature* papers. We must wait until final data comes from others.”

The erroneous 129 data landed in one of the papers, contrary to press reports, Wakayama says. (See below.) The data indeed do not affect the papers’ main thrust, but have spurred calls for closer re-evaluations of other STAP stem cell lines. Wakayama has been analyzing other lines. So far, he says, "only the 129 mouse data are not true."

In the Beginning

On January 29, a team from two prestigious research hubs—Japan’s Riken Institute, and the US’s Harvard University—published two *Nature* [1] papers finding [2] that mere coffee-mild acid could cause mature, one-week-old mouse CD45+ blood cells

to dedifferentiate to an extraordinary totipotent stem cell state. The resultant cells were called Stimulus Triggered Acquisition of Pluripotency (STAP) cells.

But in ensuing weeks, blogger scientists—notably Juuichi Jigen [3] in Japan, Paul Knoepfler [4] in the US, and anonymous PubPeer [5] commenters--have uncovered problems with the papers. Four investigations were launched by Riken, Harvard, *Nature*, and the university that approved the PhD thesis of first author Haruko Obokata, Waseda. The most serious problems have included irreproducibility, and two sets of staining images that were supposed to prove pluripotency—but instead appeared to come from Obokata's unrelated thesis, likely rendering them useless.

Genetic Analysis

Then, a few days ago, Wakayama finished a genetic analysis of two batches of STAP stem cells he made from STAP cells Obokata said came from the 129 mouse strain, and discovered another problem. For the papers, Wakayama had asked Obokata to make STAP cells from 129 mice so he could be sure the ability to dedifferentiate was not confined to the papers' main mouse strain (B6), he says.

Wakayama's main role in the project was to test, for pluripotency, STAP cells given him. Wakayama is the first scientist to clone mice. Once at Riken, he is now at Yamanashi University.

Obokata gave him two batches of STAP cells she said she made from 129 cells. But recent developments led him to analyze the stem cells he developed from them. They didn't come from 129.

"In my field, it is very important to show the same results in different strains," Wakayama said via email. "For example, cloned mice can be easily generated from F1 mice. But it is too difficult to clone from many inbred strains, such as B6—except for the 129 mouse. "

The 129 strain was briefly mentioned, in both the first *Nature* paper, and the March 5 revised protocol [6] of Riken's Hitoshi Niwa (another respected stem cell scientist and co-author). In both, it was noted "reproducible establishment" of STAP cells occurred with B6, F1, *and* 129. "STAP stem cells with all these genetic backgrounds formed chimaera-forming activity," both read.

The latter sentence seems to be an additional, if simple, "mistake," Wakayama said via email. He made stem cells from the STAP cells she said came from 129 mice. But he never made chimeras from the 129 cells, as stated in the paper (and Niwa protocol). "I gave 129 mice to Dr. Obokata. Then, Dr. Obokata generated STAP cells from those mice, and gave me two STAP cells. I do not know how many STAP cells were generated at that time. I got just two. Then, I established two STAP stem cell lines from those two STAP cells. However, I did not make chimera from those cell lines. Then I left RIken." In another email he clarified: "I left Riken one year ago. Then we could not (establish) contact well between authors. Maybe Dr. Sasai, who wrote the papers, thought that I did chimera experiment of 129."

Yoshiki Sasai, the current CDB deputy director and another respected co-author, did not immediately respond to a request for comment. Nor did Obokata.

The apparent invalidation of the report that 129 cells generated some STAP cells necessitates genetic analyses of the papers' other STAP stem cell colonies, Molecular Biology Society of Japan President Noriko Osumi told *NHK World* [7].

Osumi also told *NHK* many researchers believe STAP cells are actually embryonic stem (ES) cells. Some others think STAP culturing may select for existing stem cells.

Nishikawa, who retired as Riken deputy director months before the *Nature* papers came out, said via email that, "At this moment, I simply feel sad." But he added that CDB may want to look at "the original philosophy to establish this institute."

He did not elaborate, but Riken CDB is known for fostering innovation via a structure less strictly hierarchical than many Japanese institutes. Riken boasts many experienced basic science researchers. It also gives some bright young investigators their own labs, and more autonomy than is common in Japan. Its ten-year tenures for team leaders—another departure from Japanese career traditions--has also enabled an influx of young talent. Obokata received her own lab at 30.

Where does this leave STAP cells?

University of Hong Kong stem cell researcher Kenneth Ka Ho Lee this week [8]told *Bioscience* his lab's many failed attempts to repeat both the *Nature* protocol, and the revised Niwa protocol, have him wondering if auto-fluorescence has played a role. His group found their cells fluoresce after acid treatment. The *Nature* papers indicate this occurs because an inserted pluripotency-related Oct4-gfp reporter gene triggers fluorescence when cells dedifferentiate to stem cells. But when controls are examined, Lee sees only *auto*-fluorescence, which can occur when cells die. So an honest mistake could have been made by the Harvard/Riken crew at that stage, Lee suggests.

But that leaves a second mystery. If the cells seen by Obokata et al. were dying cells, how to explain their later contribution to all the major tissues in mouse model embryos in the papers? "What did Obokata give Wakayama to microinject into the blastocysts? Even Wakayama did not know, and I have great respect for him."

Lee is now trying the STAP team's second new protocol [9], posted last week by Harvard. He is live-blogging [10] his progress at ResearchGate. "This is getting more and more interesting—like a detective story," he concluded via email.

Next Steps

Asked about the new 129 mouse developments, a Riken spokesperson emailed: "We have received genetic analysis data on STAP stem cells from Professor Wakayama. However, this is still preliminary data, and we will cooperate with Professor Wakayama with regard to more detailed testing."

Nature's emailed statement reads: "*Nature*'s investigation into the two papers is still in progress. We have no further comment at this stage."

Dov Zipori, a Weizmann Institute dedifferentiation expert, said in an email the new problems are "terrible. All this causes huge damage that I hope would not block research in the important field of dedifferentiation."

Emailed another stem cell scientist: "What's next, I wonder?"

Links:
[1] http://www.nature.com/nature/journal/v505/n7485/full/nature12968.html
[2] http://www.nature.com/nature/journal/v505/n7485/full/nature12969.html
[3] http://stapcells.blogspot.com/
[4] http://www.ipscell.com/
[5] https://pubpeer.com/publications/24476887
[6] http://www.nature.com/protocolexchange/protocols/3013
[7] http://www3.nhk.or.jp/nhkworld/english/news/20140325_47.html
[8] http://www.biosciencetechnology.com/blogs/2014/03/nature-rejects-challenge-acid-stem-cells-scientists-try-new-tips
[9] https://research.bwhanesthesia.org/research-groups/cterm/stap-cell-protocol
[10] https://www.researchgate.net/publication/259984904_Stimulus-triggered_fate_conversion_of_somatic_cells_into_pluripotency/reviews/103