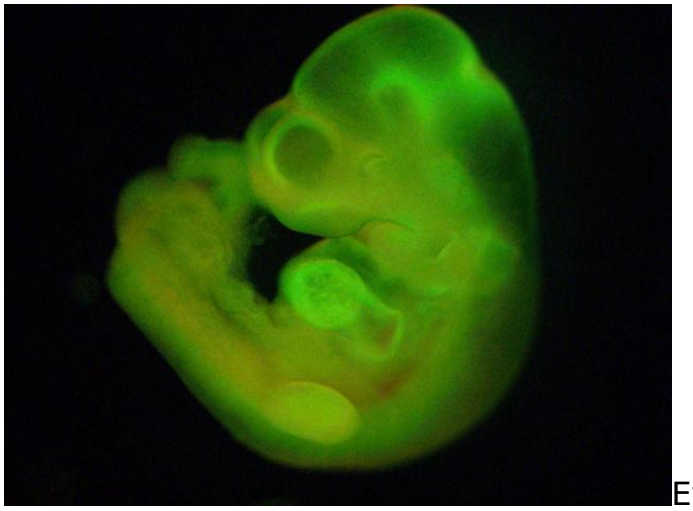
New Stem Cell Sagas

Cynthia Fox

 Even Teru Wakayama, a co-author on the *Nature* [1] reprogramming papers [2] that stunned the stem cell world this month, says he can't reach the first author on both: Haruko Obokata.

Noting this in an email to *Bioscience Technology*, Wakayama added, however, that despite many recent, anonymous claims that labs can't repeat the work—which would be historic work, if true—he is sure some eventually will.

"As you know, the work producing the first cloned animal, Dolly the Sheep, was not reproduced for a year-and-a-half until mice were cloned," said the pioneering Wakayama (who was that first [3] mouse cloner. He also was the first to clone long-dead frozen mice, and 25 generations [4] of mice from one.)

"And the first human cells cloned last year—that work [5] still has not been reproduced," he said. Wakayama added that he, himself, reproduced Obokata's work with her at the Riken Institute—but hasn't, since he moved to Yamanashi University. "Even me: I succeeded in this work at Riken, but I have not been able to in my new lab."

Still, his conclusion was firm: "I do not doubt that someone, someday, will reproduce this."

Whiplash

The news on the "new stem cells" has been whiplash-inducing all month. Bad news followed good—followed bad. In one very recent development, a Riken insider told *Bioscience Technology* this week that first author Obokata received an email from a scientist claiming to have repeated her results. Other success "anecdotes" have accumulated, the insider emailed.

But the insider warned, given many anecdotal reports of failure, it is key to stay

“agnostic.”

Then there are complaints—by scientists on the anonymous crowdsourcing site *Pubpeer*—that the papers’ raw sequencing data haven't been made public. A Riken representative said this week that is no longer true: Riken submitted the data prior to publication. “A formatting error” at first “prevented it from being deposited in the database.” But it was resubmitted on February 13, as indicated on NCBI, and is available [6].

In the midst of the confusion, lead author Haruko Obokata stopped talking to the press, including a writer [7] with *Nature*, the journal publishing her paper. But encouragingly, Riken representatives this week say her team is almost done with a much-demanded, detailed protocol, aimed at teaching all comers how to make the new stem cells. The protocol, “in the late stages of preparation,” is coming “soon” and may end much controversy. Or fuel more.

“I think I should just go to vacation in Mexico for several weeks,” co-author Charles Vacanti told *Bioscience Technology* last week.

The Beginning

It all began with two January 29 *Nature* papers. In the papers, a star Riken crew – including Wakayama and Yoshiki Sasai, deputy director of Riken’s prestigious Center for Developmental Biology (CDB)—along with Obokata and her then-lab head, Harvard University’s Vacanti, reported they dedifferentiated mature CD45 blood cells into potent, embryonic stem-cell like cells. They did it just by “stressing out” the cells a bit, serving them some coffee-weak acid.

Nature ran an accompanying news piece headlined [8]: “Acid bath offers easy path to stem cells.” The press—and stem cell world—instantly stressed out, in turn.

If any cell can transform into a stem cell easily, “this changes everything,” even the most cautious scientists were moved to say.

Accolades built. Sasai, whose precise, intuitive labwork is lionized [9] by peers, told *Nature* the papers were “amazing.” (He had come on board, to help the team with game-changing details, several months after the paper was rejected by *Nature* the first time around in 2012, says Vacanti.) This February 6, Riken—which is a top Japanese research institute—announced it would work on the cells with Kyoto University, home of Shinya Yamanaka, the Nobel-Prize winner who devised a widely praised, if complex, genetic recipe for creating pluripotent cells from adult cells (induced pluripotent stem [iPS]). (Japan has poured multi-millions into the iPS cells of Kyoto--which hosts the world’s first iPS cell clinical trial this summer.)

The Japanese press went gaga, publishing many articles praising the idea behind the *Nature* papers, often calling that idea Obokata’s. (Vacanti was actually the one to assign Obokata, a student/post doc in his lab, the task of stressing cells to dedifferentiate them. He was interested in this since postulating, in a 2001 paper [10], that pluripotent cells spied in adult tissues are recycled “phoenix”

cells--meaning, he says, cells that transform in response to harsh culturing.)

Lab after lab, worldwide, began giving acid cocktails to their cells.

But within days, the first of many complaints appeared on *Pubpeer*. (By now, there have been more than 30,000 viewings of *Pubpeer* [11] pages devoted to the papers [12]. [12]) There seemed to be many potential problems with figure and image duplication in both the *Nature* papers, and a *Tissue Engineering* paper [13] by Vacanti and Obokata in 2011. On the must-read blogsite of stem cell expert Paul Knoepfler, ten anonymous researchers claimed they could not repeat the results (if most used the wrong cells). Only days out, *Nature* reported on *itself,* reporting in a news article that ten top labs were so far unable to repeat results in its own paper.

Nature and Riken confirm they have started investigations, as has the university tendering Obokata her doctorate in 2011, Waseda. (Obokata referenced the 2011 paper in her thesis, according to a Waseda spokesperson.)

Things may be starting to shake out. Vacanti told *Bioscience* last week problems with the 2011 *Tissue Engineering* paper are limited to an image Obokata mistakenly distributed among three figures. *Tissue Engineering* plans a simple erratum for this "honest mistake," said Vacanti, who co-founded the publication. (*Pubpeer* initially made the errors public.) He also told *Bioscience* failures to repeat the work may be due to the fact the approach must vary with the cells. Tough fibroblasts respond to repeated triteration along with low pH, whereas blood cells can respond just to low pH, he said.

And Wakayama told The Asahi Shimbun *[14]* images of one mouse embryo, taken from different angles, appeared twice—once erroneously tagged as a new mouse, in the second *Nature* paper. That too was a mistake affecting no conclusions, he said. Via email, Vacanti confirms the improperly used photo [15] is in 2g. Pubpeer was the first to publicly link [16] to a Japanese blog questioning that photo.

But there are an extraordinary number of outstanding issues, the most recent brought up again by Knoepfler [17]. It is unclear when the three investigations will end. Still, many are impressed they started so quickly [18]. And most say the promised protocol will be critical.

"One of the great things about science is that it self-corrects," concludes Michael Lotze, a University of Pittsburgh oncologist studying the effects of stress on cells.

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://www.ncbi.nlm.nih.gov/pubmed/9690471
[4] http://www.cell.com/cell-stem-cell/abstract/S1934-5909(13)00008-8
[5] http://www.cell.com/retrieve/pii/S0092867413005710
[6] http://www.ncbi.nlm.nih.gov/Traces/sra/?study=SRP038104
[7] http://www.nature.com/news/acid-bath-stem-cell-study-under-investigation-1.14738
[8] http://www.nature.com/news/acid-bath-offers-easy-path-to-stem-cells-1.14600
[9] http://www.nature.com/news/tissue-engineering-the-brainmaker-1.11232
[10] http://www.ncbi.nlm.nih.gov/pubmed/11135375
[11] https://pubpeer.com/publications/24476887
[12] https://pubpeer.com/publications/1F3D9CBBB6A8F1953284B66EEA7887
[13] https://pubpeer.com/publications/20883115
[14] http://ajw.asahi.com/article/behind_news/social_affairs/AJ201402190044
[15] http://www.nature.com/nature/journal/v505/n7485/fig_tab/nature12969_F2.html
[16] https://pubpeer.com/publications/24476891
[17] http://www.ipscell.com/2014/02/are-stap-stem-cell-nature-papers-compromised/
[18] http://www.ipscell.com/2014/02/nature-post-pub-examination-of-its-own-stap-stem-cell-papers-breaks-new-ground/