"Acid Bath" Stem Cell Developments Rapidly Accumulate

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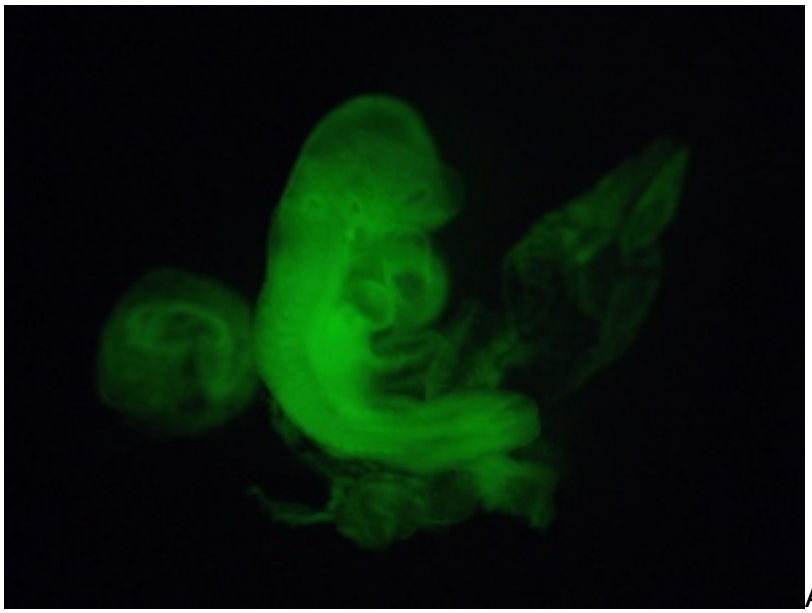 Amid plagiarism claims, the Riken Institute's Haruko Obokata reportedly told a Waseda University professor [1] she plans to withdraw [2] her 2011 thesis, a Waseda spokesperson told *Bioscience Technology* Monday morning.

Why this matters: days earlier, a global press conference revealed that a total of four (of 14) co-authors on two controversial Obokata "acid bath" papers—along with key coauthors' boss— now wanted the papers retracted mid-investigation.

Yet that same boss signaled some confidence in the *premise* of the papers, stating three times that someone internally, or externally, may repeat the work. And senior author Charles Vacanti of Harvard University told *Bioscience Technology* this morning he will not retract the papers, and will post tips as early as today to help researchers make the Acid Bath Stem Cells that Riken can no longer confirm exist.

"This is almost too amazing," said Alan Trounson, recent head of the California Institute of Regenerative Medicine (CIRM), via email Sunday night.

The Latest

The first hint that another drama was brewing hit the web at 7:24 pm Thursday night. The source was *The Asahi Shimbun* page of Facebook. "Facebook Hello," it read. "The Riken national research institute and lead STAP (acid bath) cell researcher Haruko Obokata have decided to withdraw a controversial article on the creation of STAP cells."

It was a short, eerily cheerful blurb, given the startling content. The night's upcoming press conference had been billed as an interim report, not a conclusion. Still, the news was in keeping with the tenor of the "Acid Bath Stem Cell" saga since it began on January 30, when *Nature* published two Harvard/Riken papers reporting [3] on stem cells made from mature [4] cells by simply "stressing them out" with coffee-mild acid.

At first, the papers were called historic. If we could turn any ordinary cells into extraordinary stem cells via mere mechanical stress, we could *rejuvenate* any old cells, changing the face of medicine. Labs worldwide began heating, hammering, hurling epithets at old cells. The Japanese popular press went wild over the fact she was not just a woman scientist (*rikejo*) but—as article after article trumpeted, as if making a second scientific discovery—a "beautiful" one.

But soon, labs around the world reported being unable to make the easy-to-make cells. And bloggers started turning up potential errors. They blog-jumped, lodging complaints on Japanese sites, PubPeer, ResearchGate's Open Review, the continually updated site of stem cell researcher Paul Knoepfler [5]—and back. As of Monday, there were over 68,342 views of PubPeer comments on the Acid Bath papers. Bloggers also dug up figure duplications in an earlier Obokata/Vacanti paper, forcing a recent erratum [6].

This lasted for weeks until recently, mid-way through its investigation, Riken announced it would unveil developments at a March 14 press conference.

This being the Acid Bath Sagas, the night didn't begin quietly. At about 11pm, just after the Facebook message, and before the press conference, the *Wall Street Journal* (*WSJ*) posted an unexpected article. Obokata, the elusive lead author of the papers, had just emailed the *WSJ*.

She claimed a stunning document [7] the *WSJ* and bloggers linked to—the apparent first pages of her thesis, 20 of which were near-identical to an NIH website [8]—was a "draft [9]." Until then, the thesis had been a side story. The main issue was the irreproducibility of, and potential errors in, the Acid Bath papers. But the apparent "copy and paste" thesis pages had been gaining web momentum.

Obokata wrote she had asked Waseda, where she did the thesis, to retract the "draft." But, the *WSJ* said, and *Bioscience* confirmed, Waseda Press Relations said it was unaware of the draft and the request. It was a significant development. Obokata had stopped talking, even to one of [10] her own co-authors, in February.

The Press Conference

Finally, on Thursday night (Friday afternoon in Japan), Riken held its press conference, blogged live [11] by the *WSJ*. In a room of hundreds of reporters seated at desks and and standing on tables, Nobel-Prize winning Riken president

Ryoji Noyori apologized for the papers' "grave errors," and deeply bowed. ("A big deal in Japan," said a blogger on Knoepfler's site. "Seeing Dr. Noyori bow his head actually brought a colleague of mine who used to work at Riken to tears.")

An "interim report [12]" was distributed. It noted that four co-authors were under Riken investigation: Yoshiki Sasai, a renowned and gifted stem cell biologist and deputy director of Riken CDB; Teruhiko Wakayama, a Yamanashi University cloning pioneer once at Riken; highly regarded Riken stem cell researcher Hitoshi Niwa; and Obokata.

The report said Riken was investigating six problems, including one that Riken CDB head Masatochi Takeichi called key: photos supposed to prove STAP stem cells turned into the three main germ layers of the body…but didn't. They were identical to photos from a different experiment, with different cells, from Obokata's thesis.

Noted the live *WSJ* blog, Riken CDB head Takeichi believed the papers should be retracted—even before the investigation was over. He asked the three Riken co-authors under investigation to join him in that call—and all agreed, he said. (Wakayama, approached at his new job at Yamanashi, had made the call earlier that week.) "Dr. Takeichi explains why he thinks the papers need to be retracted. The main reason, he says, is that Dr. Obokata used the same photographs she used in her 2011 doctoral dissertation in the *Nature* papers, despite the two dealing with separate studies. `It no longer stands as a research paper,' he says."

After weeks of wide-ranging speculation, there was some focus at last. In the first *Nature* paper, immunostaining images (2d and 2e)—in *in vitro* and teratoma form—were supposed to prove STAP cells form all three major germline layers, standard proofs of pluripotency. They were crucial. But they were clear copies of photos from Obokata's 2011 thesis—which was about other cells, and other experiments. So pluripotency may not have been conclusively shown.

The interim report detailed another key area under investigation: a lane in a PCR analysis (1i) that was tinkered with. It was supposed to offer evidence STAP cells are derived from mature T cells on the *starting* end. T cells have a telltale marker called a *Tcrb* gene rearrangement that withstands reprogramming. If the manipulated lane is found unusable, proof the group started with mature T cells may vanish, too.

Furthermore, an additional protocol posted online [13] by Riken March 6 found that, of eight colonies of STAP stem cells studied, none came from T cells. Even the original paper only showed the method made intermediary STAP cells—*not* full-blown STAP stem cells—out of T cells. Both the pluripotency and T cell problems reportedly [14] led Wakayama to be the first to call for the *Nature* papers' retraction, on March 10. Wakayama, whose job was to test pluripotency of cells handed him, told the *WSJ:* [15] "I myself don't know what I used in my experiments."

Still, as noted, three times Takeichi implied that while the papers have serious problems, the *idea* may not: internally and externally researchers would continue trying to make the cells, he said.

Another key point made at the March 14 press conference involved Harvard's Vacanti. When reporters asked why he was senior author on one paper, Riken's answer was that he was not heavily involved in daily cell making and analysis, but the idea of creating stem cells via injury was his [11]. This settled a misperception. Vacanti wrote about the idea as early as 2001, at the end of a stem cell paper [16]. He had assigned the task of investigating the idea to Obokata, a student in his lab.

Yet Obokata repeatedly claimed the idea was hers. In a *Nature* podcast [17] she told *Nature* interviewer David Cyranowski she got the idea "soaking in the bathtub."

She was also quoted taking credit for the idea in a Riken press release [18]. "She began to suspect that the technique she was using to *select for* pluripotent cells might instead be *creating* them," the release reads, then quotes her saying: "I'm not sure if there was a single 'Eureka!' moment, but as I kept working with and thinking about these cells, there increasingly seemed to be only one way to account for what I was seeing." (March 19 NOTE: Riken has removed the release from its site since the publication of this article. Please see a copy here [19].)

The Immediate Future

This weekend, two [1] Japanese newspapers [2] said Obokata plans to retract her thesis. A Waseda public relations officer told *Bioscience* this morning: "What's being reported is a personal email from Obokata to a professor, which reportedly mentions withdrawing the doctoral dissertation. However, as far as we know there has been no such request to the University so far."

Said Trounson this weekend via email: "It looks to me like the papers will be retracted--too many questions." But he noted the unusual faith expressed at the press conference that the papers may bear fruit regardless. "They are doing a very thorough job on the review and are keeping everyone informed. We need to wait until they complete all their investigations."

Vacanti has repeatedly told *Bioscience* his lab still easily makes STAP stem cells. (This is in contrast to Riken, where an official was reported [20] Friday saying "only the initial stages" of making STAP cells "have so far been replicated.") Vacanti clarified his role in an email Friday: "My (co-author) brother Martin (Vacanti) and I have been working on the concept of pluripotent stem cells for several years, and developed the idea to make such cells from mature somatic cells. In regard to these most recent publications, (co-author) Dr. Koji Kojima and I developed the initial experiments with Dr. Obokata, and then met regularly with her to develop, design, review and discuss the results of many of the experiments reported in the first paper. Later experiments and revisions to the manuscripts were, to a large degree, designed and overseen by our collaborators at the RIKEN, with intermittent updates given to us from Japan."

He added: “We plan to post the specific details of our most effective protocol for creating these cells on our laboratory web site to enable other investigators to replicate our findings and over time. I believe the science will speak for itself.”

This morning, he emailed that he remains "confident that what we published is absolutely correct," and will shortly post the tips.

Links:
[1] http://www.japantimes.co.jp/news/2014/03/16/national/just-stap-it-obokata-to-withdraw-her-thesis/
[2] http://ajw.asahi.com/article/behind_news/AJ201403160016
[3] http://www.nature.com/nature/journal/v505/n7485/full/nature12968.html
[4] http://www.nature.com/nature/journal/v505/n7485/full/nature12969.html
[5] http://www.ipscell.com/
[6] http://www.ncbi.nlm.nih.gov/pubmed/24625307
[7] http://stapcells.up.seesaa.net/image/Background.pdf
[8] http://stemcells.nih.gov/info/basics/pages/basics1.aspx
[9] http://online.wsj.com/news/articles/SB10001424052702304914904579438131341933464?mg=reno64-wsj&url=http%3A%2F%2Fonline.wsj.com%2Farticle%2FSB10001424052702304914904579438131341933464.html
[10] http://www.biosciencetechnology.com/blogs/2014/02/new-stem-cell-sagas-0
[11] http://blogs.wsj.com/japanrealtime/2014/03/14/live-riken-addresses-questions-on-stem-cell-research/
[12] http://www.riken.jp/en/pr/press/2014/20140314_1/
[13] http://www.cdb.riken.jp/jp/04_news/articles/pdf/14/protocol_exchange_v1.pdf
[14] http://ajw.asahi.com/article/behind_news/social_affairs/AJ201403110060
[15] http://online.wsj.com/article/BT-CO-20140310-704229.html
[16] http://www.ncbi.nlm.nih.gov/pubmed/11135375
[17] http://www.nature.com/news/acid-bath-offers-easy-path-to-stem-cells-1.14600
[18] http://www.cdb.riken.jp/en/04_news/articles/14/140130_stap.html
[19] http://www.biosciencetechnology.com/sites/biosciencetechnology.com/files/legacyimages/RIKENstapPRESSreleaseKEY.pdf
[20] http://ajw.asahi.com/article/behind_news/social_affairs/AJ201403150048