Nature Rejects Challenge to "Acid Stem Cells"; Scientists Try New Tips

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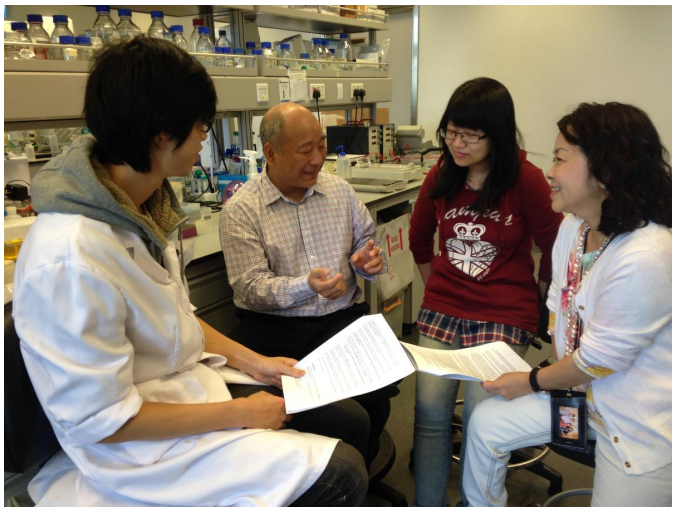

Nature has rejected the paper of a top Hong Kong stem cell researcher whose lab several times failed to replicate the results of the journal's famous "acid bath" stem cell papers—and an additional protocol [1] posted by Riken Institute co-authors weeks later.

That researcher, Chinese University of Hong Kong's Kenneth Ka Ho Lee, is trying, as of this morning, to reproduce the work as it appears in yet another new updated protocol, this time [2] posted Thursday, by the Harvard University part of the team.

"We submitted a manuscript to *Nature Brief Communications Arising* about not being able to replicate STAP, but it was rejected," Lee told *Bioscience Technology* in an email. STAP is "Stimulus Triggered Acquisition of Pluripotency." It is the name given to stem cells, described in two January 29 Obokata et al. *Nature* [3] papers, created by just stressing ordinary mature cells with mild acid. The papers were produced by a team from the US's Harvard and Japan's prestigious Riken.

The reason his manuscript was rejected, Lee said, quoting from the *Nature* letter: "'In the present case, while we appreciate the interest of your comments to the community, we do not feel that at this stage they challenge key data or conclusions of the paper by Obokata et al., and therefore we cannot offer to consider your paper for publication in our Brief Communications Arising section.'" Said a frustrated Lee, chief of the Stem Cell and Regeneration Thematic Research Programme: "What can I say? If the first step of making STAP cannot be replicated, then it `challenges key data or conclusions of the paper by Obokata.'"

Lee is nonetheless trying again, now using tips released Thursday. That protocol emphasizes trituration--stressing cells via strenuous pipette manipulation —more

than the original paper did.

It also emphasizes shifting the cells twice a day the first week, to stop adherence and differentiation, senior author Charles Vacanti told *Bioscience Technology* in interviews in recent weeks. Vacanti is a Harvard author behind the newly posted protocol.

Vacanti said his Harvard crew repeatedly, over years, created STAP cells from many mature cell types via techniques focused on robust trituration. It was around the time that first author Haruko Obokata came on board that an acid-oriented approach was tried on one-week old mouse spleen cells.

After years of “changing nutrients, trituration, and enzymes, finally by 2006 (co-author Koji Kojima) grew stem cells from lung perfectly every time,” Vacanti said. Another worker in his lab succeeded with muscle. In 2008 “that worker said, 'Yes, now it works perfectly.'” That was when Vacanti asked Obokata, who had been a student in his lab, to do “what we did with lung” to lymphocytes. After she did this, the original paper was turned down by *Nature* in April 2012. But then top researchers with Riken, where Obokata got a new job, did some "brilliant" new work, Vacanti said, and the paper was approved at the end of 2013.

But ever since Obokata et al., with its acid bath focus, was published in January, serious concerns [4] have circulated about near-universal irreproducibility [5], and duplicated images from Obokata's unrelated thesis in a key pluripotency section.

The first set of additional tips posted (by three Riken co-authors including Obokata) on March 5 raised more concerns, as they indicated no cells studied expressed the *Tcrb* marker that would prove STAP stem cells came from mature cells.

Vacanti told *Bioscience* in recent weeks a stronger emphasis on trituration--in addition to the acid--may make a difference.

Lee is giving it a try, noting that indeed “Harvard put a greater emphasis on trituration.” But, he said, he is “shocked” by the somewhat “amateurish” way it has all proceeded. The Harvard/Riken group had reported in the original papers seeing many green cells after acid baths. The reported reason: the cells, genetically altered to glow via green fluorescent protein (GFP) when the Oct4 pluripotency gene was on, had gone pluripotent.

But that may just be “auto-fluorescence,” which can occur with cell death, Lee said.

“Mistaking necrotic cells for activated Oct4-GFP expression could be an honest mistake,” Lee said. “When I first tried to repeat the STAP experiment, we did see GFP-positive cells. But after normalization of the images with our negative control cell cultures, it was false positive auto-fluorescence. We validated our observation by qPCR that the cells did not express (pluripotency genes) Oct4, Sox2 and Nanog.”

Lee said he is also worried about the original study's "tetraploid" embryo generation assay. If the above honest mistake was made, and the team mistook auto-

fluorescence for pluripotency, why was that assay positive for *totipotent* cells?

The groups had reported in their original papers that their cells were more than pluripotent—they were totipotent. Totipotent cells (creating both embryo *and* placenta) have not been isolated before. The above assay involves injecting GFP-marked cells into a blastocyst. If green cells appear in every tissue type except placenta, the starting cell was pluripotent. It is another story if they appear in all tissue types *including* placenta.

“To generate embryos from the STAP cells is my major concern,” said Lee. “What did Obokata give (Teru) Wakayama (the papers' embryo generator) to microinject into the blastocyst ? Even Wakayama did not know, and I have great respect for him. (Normal pluripotent) embryonic stem (ES) cells that constitutively express GFP generate GFP-positive chimeras. If injected into tetraploids, a green embryo will be generated. But the placenta will still be GFP negative. For both the embryo and placenta to be positive, this could only be possible if embryos were extracted from homozygous GFP pregnant mice.”

Lee concluded, mystified: “If STAP cells do not exist, the only way a green embryo with a green placenta can form is if the embryo was just extracted from a non-manipulated GFP pregnant mouse.” But, he said firmly: “I am not claiming the authors are fraudulent. The above is only based on the context that STAP cells do not exist.”

The original paper aside, Lee is not alone in his initial wariness of the newest protocol. One ES cell expert told *Bioscience* the vigorous trituration seems too harmful to cells, and hard to do.

Cancer stem cell researcher Hasan Korkaya of Georgia Regents University said in an email: “I think for Harvard to put out the protocol is a step in the right direction, but it all still depends on the reproducibility of the data by independent investigators. And we actually do triturate normal mammary cells when we isolate them from reduction mammoplasties. If that turns them into stem cells, theoretically we should end up with all stem cells. But that is simply not the case. We do not get more than five percent or so mammary stem cells.” (Note: the papers' published "acid bath" stem cell rate was at eight percent.)

A developmental immunologist emailed that “there seems to be a lot of places for things to go wrong. I’d hate to try and repeat this protocol. It’s full of twitchy things that simply might not work without any idea why.”

But others held out hope, noting that other studies indicate many stressors may indeed cause cells to retreat to a stem cell state. Weizmann Institute stem cell researcher Dov Zipori is coming out with a *Stem Cells* paper finding adult mesenchymal cells can dedifferentiate via the simple stress of isolation to the single-cell level. He sees parallels. “The Vacanti protocol indicates again that the different ways he stresses the cells cause huge cell loss -- leading to dilution and separation from partner cells.”

Will heavy trituration make the difference? The next two weeks may bring the final answer. Lee's group will post its progress for all researchers to follow on his ResearchGate [6]site, where he also posted a detailed explanation of his lab's past attempts to reproduce Obokata et al.

Links:
[1] http://www.nature.com/protocolexchange/protocols/3013
[2] https://research.bwhanesthesia.org/research-groups/cterm/stap-cell-protocol
[3] http://www.nature.com/nature/journal/v505/n7485/full/nature12968.html
[4] http://www.biosciencetechnology.com/blogs/2014/03/acid-bath-stem-cell-developments-rapidly-accumulate
[5] http://www.ipscell.com/stap-new-data/
[6] https://www.researchgate.net/publication/259984904_Stimulus-triggered_fate_conversion_of_somatic_cells_into_pluripotency/reviews/103