NATURE NEWS BLOG

Acid-bath stem-cell team releases tip sheet

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A group of Japanese researchers whose revolutionary method to produce stem cells simply drew questions from other biologists has published more details of their protocol.

The authors, who developed an 'acid-bath' technique that others have so far been unable to reproduce, released technical tips with a press statement today and published them on Nature Protocol Exchange. The document is entitled 'Essential technical tips for STAP cell conversion culture from somatic cells'.

In it, Haruko Obokata, Hitoshi Niwa and Yoshiki Sasai, all of the RIKEN Centre for Developmental Biology in Kobe, say that despite its "seeming simplicity", the method requires special care. But it is "absolutely reproducible", Niwa told *Nature* News.

The controversy began at the end of January when Obokata and colleagues released two papers in *Nature* detailing how stress — in the form of low pH or physical pressure — could trigger the reprogramming of a mouse's cells into an embryonic state, a process they called stimulus-triggered acquisition of pluripotency (STAP).

Cells reprogrammed into this state are ideal for studying the development of disease or the effectiveness of drugs, and could also be transplanted to regenerate failing organs. Making another type of pluripotent stem cell, called induced pluripotent stem (iPS) cells, requires a complex recipe of chemical or genetic factors. Obokata's simple technique made headlines around the world.

But after the papers were published, they came under attack for a number of reasons, including the presence of duplicated images, an apparently plagiarized passage and the abnormal presentation of certain data. This led some commenters to question the validity of the results.

Something that would resolve the controversy would be the replication of the results by another group, but so far there have only been reports of failed attempts.

Despite a media frenzy, especially in Japan, with headlines even suggesting the results are fraudulent, the authors have stood by the work. Today Niwa told *Nature* News that members of the team besides Obokata have replicated the bulk of the work and that others outside the laboratory have succeeded in the first crucial step, inducing Oct3/4 expression after the acid treatment.

But the authors admit that the procedure is more complicated than originally advertised, leading to the publication of the tips.

The 10-page document states: “Despite its seeming simplicity, this procedure requires special care in cell handling and culture conditions, as well as in the choice of starting cell population.” The authors also point to the importance of bringing the cells gradually to the brink of death — which kills some 80% of them after 2–3 days — to reach the “optimal level of sub-lethal stress”.

The tips break down the process into three sections: collection of tissue and treatment with low-pH needed to produce STAP cells; preparing the culture needed to convert STAP cells to STAP stem cells, which behave like iPS cells or embryonic stem cells; and preparing the culture needed to turn STAP cells into “FI cells”, which can form placenta.

The document includes 28 “important” tips, which note the necessity of starting with primary cells (as opposed to cultured cells); that mice less than a week old, especially male mice, gave better results; the recommendation of using non-adhesive plates, which allow cell mobility and cluster formation; the importance of getting the cell density right in culture; the recommendation of using mice of a specific genetic lineage and many other detailed hints for using the proper culture conditions.

Martin Pera, a stem-cell researcher at the University of Melbourne in Australia, says: “The details provided in Nature Protocol Exchange will undoubtedly be helpful to those trying to repeat these findings.” But Pera, who has not tried to make STAP cells, adds: “The additional information does not seem to me to reveal any key procedural detail without which it would be impossible to duplicate the work. It appears instead to reinforce and emphasise some aspects of the technique that were disclosed originally.”

Those who are trying to replicate the method are intrigued by the publication of the tips. Jacob Hanna, at the Weizmann Institute of Science in Rehoboth, Israel, has made 10 batches of cells in an as-yet-unsuccessful effort to make STAP cells. He looks forward to trying some of the tips on culture conditions. “Some protocols can indeed be tricky and finicky and I commend the authors on making the effort to reach out to the scientific community,” he says. But he questions how the more complicated protocol would apply to another method of producing STAP cells advertised in the original article — putting pressure on the cell membranes. ”I find that hard to imagine as a very complicated manipulation,” he says.

Qi Zhou, of the Institute of Zoology in Beijing, also appreciates the authors sharing all the details, as “some were overlooked” in his efforts to make STAP cells. The restrictions on the origin of the cell type to specific stage and gender “raise very interesting questions which may help to explain the underlying mechanism of STAP”, he says.

Niwa says that the original team is working on a “full protocol” that will make it easier to make STAP cells, but that won’t be available for at least a month. “We are not sure when it will happen because we are now trying to improve some point to enhance the reproducibility,” he says.

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Comments

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Jay Schufman said: Unfortunately, this sounds more like alchemy than science! If it is science, then it should be reproducible by following a set procedure, and that procedure should be elegant, not crouched in symantics and mysticism! (There's one born every minute!)

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