April 1, 2014

Re-evaluation of the reported STAP phenomenon

Under the direction of RIKEN President Ryoji Noyori, RIKEN will implement a research program to re-evaluate the reported STAP (stimulus-triggered acquisition of pluripotency) phenomenon.

I. Experimental plan

- Overview

We will adopt a strictly rigorous scientific approach to re-evaluation of the existence or non-existence of the reported STAP (stimulus-triggered acquisition of pluripotency) phenomenon. This will involve not only examining whether induction of pluripotency in lymphocytes, as described in the published reports, is reproducible, but also the use of cell lineage tracing technology to stringently assess whether this phenomenon can be replicated in other types of differentiated cells as well. These experiments will also involve the use of blastocyst injection of generated cells, if any, to assess their ability to contribute to chimera formation, which is a highly rigorous method for the evaluation of pluripotency. A detailed experimental plan follows.

Assessment of the reproducibility of pluripotency induction in lymphocytes (cultured cells)

(1) We will follow the recommendations described in the document "Essential technical tips for STAP cell conversion culture from somatic cells" (published on March 5, 2014; hereafter, "technical tips"), and collect spleen tissue from neonatal transgenic Oct3/4 GFP-expressing mice within one week of birth to obtain $CD45^+$ blood lineage cells. These cells will be treated with low pH, cultured for seven days, and assessed for induction of GFP expression. In the event that STAP-like cell clusters expressing GFP are observed, they will be screened for the expression of pluripotency marker genes.

(2) In the event that the process described in (1), above, yields results suggesting the reproducibility of the reported phenomena, we will evaluate the following:
   a. The STAP-like cell clusters will be subdivided and injected into blastocysts; assessment of chimeric contribution will be made using T cell receptor (TCR) rearrangement as the primary index.
   b. STAP-like cell clusters will be differentiated and cultured following the methods described in the technical tips, and evaluated for their ability/inability to generate STAP stem cells. In the event that STAP stem cell-like cells are obtained, they will be assessed for TCR rearrangement using PCR, and their pluripotency will be evaluated by blastocyst injection.
   c. During the re-evaluation process, the methods described in the current technical tips will be tested to determine whether they can be more rigorously optimized to increase the reproducibility of the reported findings.

Stringent test of the induction of pluripotency in differentiated cells (mouse experiments)

(1) We will cross transgenic mice in which Cre recombinase is expressed in cell-type-specific manner, with transgenic mice in which Cre recombinase-expressing cells are labeled by a constitutive genetic marker, to obtain neonatal double-transgenic pups up to one week after birth. (Cre-loxP system)

(2) We will extract organs containing labeled cells from mice described in (1), above, and subject isolated individual cells to low-pH treatment, culture them for seven days, and assess for formation of STAP-like cell clusters carrying fluorescence-labeled genes indicating a differentiated cell origin.

(3) In the event that the processes described in (1) and (2), above, yield results suggesting the reproducibility of the reported phenomena, we will evaluate the following:
   a. The STAP-like cell clusters will be subdivided and injected into blastocysts; assessment of chimeric contribution will be made using fluorescence-labeled genes.

b. STAP-like cell clusters will be differentiated and cultured following the methods described in the technical tips, and evaluated for their ability/inability to generate STAP stem cells. In the event that STAP stem cell-like cells are obtained, they will be assessed for expression of fluorescence-labeled genes, and their pluripotency will be evaluated by blastocyst injection.
c. During the re-evaluation process, the methods described in the current technical tips will be tested to determine whether they can be more rigorously optimized to increase the reproducibility of the reported findings.

2. Organization

- Project supervisor
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  Senior Advisor/Head of Aizawa Research Unit, RIKEN Center for Developmental Biology

- Research coordinator
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  Project Leader, Laboratory for Pluripotent Stem Cell Studies, RIKEN Center for Developmental Biology

3. Schedule

The re-evaluation effort will begin on April 1, 2014 and is projected to require approximately one year to completion. The Project Supervisor will submit periodic progress reports to the RIKEN Board of Directors. Based on the reported results, the Board of Directors will determine whether to continue the re-evaluation experiments.

4. Public information

An interim report will be released approximately four months after the start of the project, and a final report will be released at the conclusion of the project.

II. Support for independent evaluation efforts

RIKEN will endeavor to respond to all inquiries from researchers outside the institute regarding the experimental protocol. In the event that better optimized methods than those described in the current technical tips are discovered during the course of the re-evaluation experiments, we will make that information public immediately, and may hold training programs in our efforts to support further re-evaluation efforts and research.