March 31, 2014

To: Ryoji Noyori, RIKEN President

Report on STAP Cell Research Paper Investigation

Research Paper Investigative Committee
Shunsuke Ishii, Chair
Atsushi Iwama
Haruhiko Koseki
Yoichi Shinkai
Tetsuya Taga
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1. Circumstances

On Thursday, February 13, 2014, a RIKEN researcher who had been notified of doubts concerning research papers published by RIKEN scientists contacted the RIKEN Auditing and Compliance Office through one of RIKEN's executive officers. The director of the Auditing and Compliance Office decided that this matter should be handled in compliance with the provisions for reports on research misconduct stipulated in Article 10, paragraph 3 of RIKEN's Regulations on the Prevention of Research Misconduct (September 13, 2012, Reg. 61, hereafter "Regulations"), and from that same day through February 17, the Office conducted a preliminary inquiry, in accordance with the provisions of Article 11 of the Regulations. This preliminary inquiry was carried out by the following five people: Shunsuke Ishii, Atsushi Iwama, Haruhiko Koseki, Yoichi Shinkai, and Tetsuya Taga. In response to the results of this preliminary inquiry, RIKEN decided to carry out a full investigation as stipulated in Article 12 of the Regulations, and an Investigative Committee was established on February 17, with Shunsuke Ishii serving as Chair.

2. Methods and contents of the investigation

2-1. Purpose of the investigation and items investigated

The investigation sought to clarify whether or not the following items constituted "research misconduct" as defined in Article 12, paragraph 2 of the Regulations.

(1) Obokata, et al, Nature 505:641-647 (2014) (Paper 1)

- (1-1) Unnatural appearance of colored cell parts shown by arrows in d2 and d3 images of Figure 1f.
- (1-2) In Figure 1i, lane 3 appears to have been inserted later.
- (1-3) A part of the Methods section on karyotyping appears to have been copied from another paper.
- (1-4) A part of the procedures described in the Methods section on karyotyping appears to be different from the actual procedures used in the experiment.
- (1-5) The images for Figures 2d and 2e appear to be incorrect, and closely

resemble images in Dr. Obokata's PhD dissertation.

Haruko Obokata (lead author, corresponding author), Yoshiki Sasai (co-author), Teruhiko Wakayama (co-author), and Hitoshi Niwa (co-author)

(2) Obokata, et al, Nature 505: 676-680 (2014) (Paper 2)
  (2-1) There is a strong resemblance between the rightmost panel in Figure 1b and the lower panel in 2g, both showing fluorescence in mice placenta.

Haruko Obokata (lead author, corresponding author), Yoshiki Sasai (corresponding author), Teruhiko Wakayama (corresponding author), Hitoshi Niwa (co-author)

2-2. Individuals investigated

The individuals investigated held the following positions at the time that papers 1 and 2 were submitted and when they were published.

Haruko Obokata
At the time of submission: Research Unit Leader of the Laboratory for Cellular Reprogramming, RIKEN Center for Developmental Biology
At the time of publication: Same as above

Yoshiki Sasai
At the time of submission: Group Director of the Laboratory for Organogenesis and Neurogenesis, RIKEN Center for Developmental Biology
At the time of publication: CDB Deputy Director

Teruhiko Wakayama
At the time of submission: Team Leader of the Laboratory for Genomic Reprogramming, RIKEN Center for Developmental Biology
At the time of publication: Professor at the Faculty of Life and Environmental Sciences, University of Yamanashi, and Senior visiting Scientist at RIKEN

Hitoshi Niwa
At the time of submission: Project Leader of the Laboratory for Pluripotent Stem Cell Studies, RIKEN Center for Developmental Biology
At the time of publication: Same as above

2-3. Investigation methods

From February 20 through March 31, 2014, the Investigative Committee collected and examined the relevant materials and conducted interviews with the individuals concerned.

The materials included the original data of the experiments described in the papers, lab notes, files showing the process of creation of the papers, documents provided by the individuals investigated, emails exchanged among the individuals concerned, and equipment that was used in the experiments.

In addition, opinions regarding the reconstruction of the imaging data were solicited from Professor Akihiko Nakano, Laboratory of Developmental Cell Biology, Department of Biological Sciences, Graduate School of Science, University of Tokyo, who is also Team Leader of the Live Cell Molecular Imaging Research Team, Extreme Photonics Research Group, RIKEN Center for Advanced Photonics, and an authority on imaging.

The Investigative Committee based its inquiry on examinations of these materials

and interviews.

2-4. Results of the investigation and opinions

(1-1)	Paper 1: Unnatural appearance of colored cell parts shown by arrows in d2 and d3 images of Figure 1f. (*Investigation results already covered in interim report)*

Results of investigation

Dr. Obokata stated that she performed the live imaging, from which the still images published in the paper were made, that she submitted these as compressed images, that the original images in the submitted manuscript contained no distortion, that she did not notice the presence of distortion in the published images, and that she does not know why such distortions were generated.

The submitted original live imaging data were examined. Upon reproducing the images on several computers, it was confirmed that the images submitted with the manuscript contained no distortion, while the images in the published papers contained some distortion.

Dr. Akihiko Nakano explained the possible causes of the distortion as follows. Although it was not possible to reproduce from the submitted live imaging still images identical to those in the paper, very similar images were created. Distortions result when the resolution is decreased and the images are compressed using JPEG or some other method. Reproducing the same distortion is difficult, because it depends on the degree of the compression. Therefore, if the distortions were generated in the process of figure preparation at the *Nature* editorial office, it is difficult to accurately reproduce those distortions. It is possible, along with compression, for block noises to be generated that could cause the appearance of colors that are not in the original image. Given these reasons, it can be concluded that the published images constitute single frames captured from the live imaging.

Opinions

It is reasonable to conclude that the still images published in the papers were generated from the submitted live imaging. The images in the submitted manuscript contained no distortions, but distortions are evident in the published images. It is plausible these distortions were produced during figure processing at the *Nature* editorial office. Block noise, which can be generated during compression, is a widely known phenomenon. Therefore, it is judged that there was no falsification in the process of generating the images in question from the live imaging.

(1-2)	In Figure 1i, lane 3 appears to have been inserted later.

Results of investigation

Drs. Obokata and Sasai submitted an electronic file of the photos of the gels on which Figure 1i is based, lab notes, and a written explanation of the process and methods used to create the figure. The two were also interviewed separately.

After careful review of all of the information acquired, it was confirmed that Figure 1i is a processed image of 2 photos taken of 2 pulse-field electrophoresed gels. There were a total of 29 samples, with samples 1 through 14 electrophoresed on gel 1 and samples 15 through 29 on gel 2. It was confirmed from the photos of the two gels that lanes 1, 2, 4, and 5 of Figure 1i correspond to lanes 1, 2, 4, and 5 of gel 1, counting from

the left (standard DNA size marker is lane 0 on the left), and lane 3 corresponds to lane 1 of gel 2 (standard DNA size marker is lane 0 on the left). Lane 3 of gel 1 and lane 1 of gel 2 were both positive controls indicating the rearrangement of T-cell receptor genes, and were, respectively, electrophoresed PCR products of $CD45^+$ hematopoietic cell and $CD45^+/CD3^+$ T lymphocyte DNA.

Regarding the image processing, it was confirmed that in the gel 1 photo of lanes 1, 2, 3, 4, and 5, lane 1 of gel 2 was not simply inserted in the location of gel 1's lane 3. The separation distance of the standard DNA size marker lane in gel 1 is approximately 0.63 times that in the latter gel 2. In preparing Figure 1i, the image of gel 1 was vertically elongated approximately 1.6 times before inserting the image of gel 2's lane 1. This was confirmed by the vertical warping seen in the images of dust in gel 1. A light smear in the photo of gel 2's lane 1 appears to have been erased, suggesting that contrast adjustments were also made.

When Dr. Obokata was queried on this, she explained that lane 1 of gel 2 was the most suitable for clearly showing the rearrangement of T-cell receptor genes as a positive control. She stated that after visually confirming that the log-scale values of molecular weight and separation distances for the standard DNA size markers had satisfactory linearity in their respective gels, she vertically elongated the photo of gel 1 and decided on the location for the insertion of lane 3 based on the location data for the standard DNA size marker. Upon verification, it was found that there was no linearity between the log-scale values of molecular weight and separation distances of the standard DNA size markers for gel 1 and gel 2, and that it would have been impossible to position lane 3 on the basis of the standard DNA size marker location data, as had been explained. In addition, her explanation was not supported by the fact that even if the image of lane 3 was positioned in conjunction with the standard DNA size markers located near the T-cell receptor gene rearrangement band group of lane 3 in Figure 1i, the T-cell receptor gene rearrangement band group would be placed differently from the T-cell receptor gene rearrangement band shown in lane 3 of Figure 1i. Contrary to her explanation, if the image in lane 3 is positioned in reference to the position of the T-cell receptor gene rearrangement band group in lane 3 of Figure 1i, a discrepancy appears between the positions of the standard DNA size marker bands in gels 1 and 2. As a result, this suggests that when Figure 1i was processed, it was not the standard DNA size marker bands that were taken as the standard, but rather the lane was inserted to fit with the shape of the T-cell receptor gene rearrangement band group in the adjacent lane 4.

With regard to the electrophoresed samples, the information provided by Dr. Obokata, including sample tube labels and the lab notes, indicated that lanes 1, 2, 4, and 5 in Figure 1i are consistent with the paper, and that the "Lymphocytes" label for lane 3 refers to $CD45^+/CD3^+$ T lymphocytes.

<u>Opinions</u>

It is clear from the detailed analysis of the figure in question that it is a composite image created from two separate images of the electrophoresed gels. The composite was created by deliberately manipulating—in what can hardly be called a minor way—images of multiple lanes including the two corresponding to FACS-Sorted Oct4-GFP positive cell group samples which play an important role in this paper. An image of these lanes had been vertically elongated approximately 1.6 times, in which a positive control lane from the other gel was placed after contrast adjustment. Furthermore, in placing the positive control lane, no scientific considerations were made nor scientifically reasonable procedures were followed; instead, the lane for the T-cell receptor gene rearrangement band group was positioned on the basis of visual confirmation. This not

only created the illusion that the data of two different gels belonged to only one gel, but may also lead to the danger of misinterpretation of the data.

It would appear that Dr. Obokata did not, at that time, sufficiently understand the prohibitions against the action that she had taken, nor did she appear to know *Nature*'s criteria for presenting such data in a way that would not call its authenticity into question. Even though her direct intent may not have been to deliberately mislead other researchers or lead them to incorrect interpretations of the data, our conclusion is that she was aware of the danger. It is evident that her purpose in creating the composite image was to articulate the T-cell receptor gene rearrangement band and that she did so without applying scientific consideration or procedures. We therefore conclude that this was an act of research misconduct corresponding to falsification.

The falsified image was created by Dr. Obokata using her own experimental data. Drs. Sasai, Wakayama, and Niwa had no involvement in the experiments or in creating the image data. The three were shown the already altered image prior to the submission of the paper to *Nature* without being told that it was a false image. Given that this alteration could not be easily detected, it must be concluded that there was no research misconduct on the part of these three researchers.

(1-3) A part of the Methods section on karyotyping in Paper 1 was found to have been copied from
Guo J., et al.; Multicolor Karyotype Analyses of Mouse Embryonic Stem Cells. in In Vitro Cell Dev Biol Anim 41(8-9), 278-283 (2005), and this also was investigated.

(1-4) A part of the procedures described in the Methods section on karyotyping appears to be different from the actual procedures used in the experiment.

Results of investigation

Regarding (1-3)

Dr. Obokata explained that in the Genomic Reprogramming Research Team under Dr. Wakayama, karyotyping was carried out on a day-to-day basis, but that the protocol used was a very simple one, and deciding that a more detailed explanation was needed, she referred to a paper that explained the protocol in detail, but forgot to include a note. She confirmed that she wrote the Methods section, and while she seemed to vaguely remember copying some part of it, she did not have a copy of the paper from which it was copied, and did not remember the source. The similarity of the text, the fact that Dr. Obotaka was not familiar with the protocol, and that the description in the paper does not correspond exactly to the procedures followed in the actual experiment, lead to the conclusion that the text was somehow copied from the Guo paper.

Regarding (1-4)

Drs. Wakayama and Obokata, and the staff who carried out the experiment, all explained that the karyotyping was carried out by Dr. Wakayama's staff, and that he gave the data to Dr. Obokata. The preparation of the cell sample was carried out in line with the explanation in the Methods section, but Dr. Wakayama explained that his staff carried out the hybridization and imaging using Applied Spectral Imaging's SKY FISH system, which was different from what was written in the Methods section. The image

files, including creation date information, were submitted. Dr. Wakayama explained that this section under Methods had been written by Dr. Obokata, and that she did not know the details of the experiment with hybridization and imaging.

Opinions

Regarding (1-3)

The Paper 1 Methods section in question consisted of 17 lines copied from the paper by Guo J., et al., without citing the source. This is absolutely not allowed, and this is something that is strictly taught at research institutions and universities. Appropriate quotation and citing of all sources is a matter of course for all researchers. Dr. Obokata's explanation that she did not possess a copy of the Guo paper, did not remember where she had copied the text from, and that it was simply oversight, is highly questionable.

Still, given the content of the text and the volume that was copied, the failure to cite the source cannot automatically be judged to have been done deliberately. Dr. Obokata correctly cites her sources in 40 places in the main text of the paper and in one other place in the Methods section. The Methods section in question is the only place where she fails to do so. Karyotyping is commonly carried out in many laboratories, and the same procedures are generally used. If Dr. Obokata was unfamiliar with the procedures, her explanation that Dr. Wakayama's protocol was a very simple one, and that she therefore searched for a more detailed explanation, is understandable. Given that the text she found provided an explanation of generally carried out procedures, it is not so irrational that she has no memory of where she got the text from. Likewise, the fact that she did not possess a copy of the Guo paper does not contradict her explanation that she merely forgot to cite the source. Her lack of memory on this point makes it impossible to conclude that her failure to cite the source constitutes misconduct.

Regarding (1-4)

Dr. Obokata failed to check the accuracy of her description with those who actually carried out the procedures or with Dr. Wakayama or the other co-authors. The co-authors also failed to check this section carefully before the paper was published. It is evident that some of the procedures in the description are different from what was actually carried out, but this cannot be judged as research misconduct.

The inaccuracies of (1-3) and (1-4) can be judged as the result of oversight, but a scientist has an obvious obligation to accurately record experimental procedures, and proper citation of quoted text is fundamental. Plagarism is unacceptable.

This experiment was carried out by Dr. Wakayama's staff, and the inaccuracy of the text could have been easily corrected if Dr. Wakayama had carefully checked this portion of the paper, and in this respect he bears responsibility. Still, his failure to detect Dr. Obokata's error was simple oversight, not research misconduct. There is no judgement of misconduct for Drs. Sasai and Niwa as they were not involved in this experiment.

(1-5)	The images for Figures 2d and 2e appear to be incorrect, and closely resemble images in Dr. Obokata's PhD dissertation.

Results of investigation

On February 20, the committee was presented by Drs. Sasai and Obokata with a request for correction and with supporting documentation. They brought up two points: One was that some of the immunofluorescence images of in vitro differentiated cells and teratoma (the central image in the bottom row of Figure 2d and the three images in the

bottom row of Figure 2e), actually were derived from STAP cells created out of bone marrow hematopoietic cells but not spleen hematopoietic cells; and the second point was that they were thinking of replacing the incorrect images. The supporting documentation they provided consisted of these image files. Dr. Obokata explained that she mistook the images because both the spleen and bone marrow hematopoietic cell samples had the same “hemato” (hematopoietic) label.

Later, it was discovered that the images in Paper 1 very closely resembled images she had used in her doctoral dissertation for Waseda University. At the time the request was made for a correction, however, it was not explained that the images had come from Dr. Obokata’s doctoral thesis. Both Dr. Sasai and Dr. Obokata said that they had thought it was allowable to use images from a doctoral thesis for a paper to be submitted to an academic journal and that there was therefore no need to explain that this is what had been done.

Paper 1 presents STAP cells created by subjecting the spleen cells of a 1-week old mouse to an acid bath, while in Dr. Obokata’s doctoral thesis she describes acquiring “sphere” cells (sphere-shaped cell clusters) by forcing the bone marrow cells of a 3 to 4-week old mouse through a narrow pipette in a process of applying mechanical stress to the cells. The two experimental conditions are quite different. Dr. Obokata claims not to have sufficiently recognized the difference between the two experiments and to have made a simple mistake in using the wrong images. An analysis of the images in Paper 1 confirmed that they were copied from a similarly positioned figure in her doctoral thesis. It was also found that the images in Paper 1 were the same as those in the paper that was submitted to *Nature* in April 2012 and which had been rejected. It was confirmed that in this rejected paper, there were 3 immunofluorescence images of differentiated cells from the “sphere” cells acquired through the mechanical stress procedure outlined in Dr. Obokata’s doctoral thesis, 3 hematoxylin and eosin stained images of teratoma generated by the “sphere” cells, as well as 3 more immunofluorescence images of teratoma, all closely resembling images in her doctoral thesis. When she later resubmitted her paper to *Nature*, Dr. Obokata replaced some of these images with those of STAP cells acquired through the acid treatment, but she explained that even then she did not notice the other incorrect images. We attempted to trace the source of the image data by looking through her lab notes, but there were only two notebooks covering a span of three years, and these contained so little detail that it was impossible to scientifically trace the source of the image data.

Dr. Sasai was informed of the incorrect images by Dr. Obokata only a few days before the February 20 interviews with the investigative committee. He explained that he immediately instructed Dr. Obokata to prepare accurate image data so that the submitted paper could be corrected. The immunofluorescence images of teratoma that were submitted to replace the incorrect images were created on February 19. Both Dr. Sasai and Dr. Obokata expressed deep regret for not telling the investigative committee that material had been used from a doctoral thesis, explaining that they had assumed it was permissible to do so, and that they did not think an explanation was necessary because they had been able to produce the correct images for replacement.

Opinions

It was determined that Dr. Obokata had used images in Paper 1 that very closely resembled images in her doctoral thesis. Yet the experimental criteria for the two papers were different. The core message of Paper 1 was that a very easy method using an acid bath had been discovered. It is hard to believe that Dr. Obokata was unaware of the different experiment conditions when she prepared the images. Also, there are traces around the images in Paper 1 that suggest they were cut out of an identical

arrangement of images in the doctoral thesis. This makes it very difficult to accept Dr. Obokata's assertion that she cut and pasted the images from the thesis to Paper 1 without realizing that they represented completely different experimental procedures. Still, it was found that data was handled extremely carelessly, so it is possible that data of unknown origin that could not be verified or traced scientifically was used in the submitted paper. Regardless, this data was extremely important in showing the pluripotency of the STAP cells, and the actions taken by Dr. Obokata completely undermine the credibility of the data. There is no doubt that she was fully aware of this danger, and we therefore conclude that this was an act of research misconduct involving fabrication.

Dr. Obokata carried out experiments to produce teratomas when she was working in Dr. Wakayama's laboratory as a visiting researcher, and later as the head of her own laboratory. As a laboratory head and the supervisor of these kinds of experiments, Dr. Wakayama had a responsibility to check the validity and accuracy of the data and to ensure that all data were handled properly. Dr. Sasai, also, was substantially involved in overseeing the writing of the paper, and was therefore equally responsible for confirming the validity and accuracy of the data. They were negligent in allowing this kind of fabrication, but though this does not extend to confirmation of their direct involvement in the fabrication, they still bear heavy responsibility given their standing. Dr. Niwa, on the other hand, did not become involved until at a very late stage in the paper preparation, and is therefore not considered to have been involved in research misconduct.

It is to be noted that the original explanations about the mistaken images by Dr. Sasai and others were insufficient. This failure endangered the accuracy of the investigation and an honest response was called for.

(2-1)	Paper 2: Regarding the images of fluorescence in mice placenta, the rightmost panel in Figure 1b is very similar to the lower panel in Figure 2g.

Results of investigation

Dr. Wakayama explained that these were two photos of the same chimera mouse generated from STAP cells, taken from different angles by Dr. Wakayama himself. He explained that he handed them to Dr. Obokata as electronic files after labeling them and including them with other chimeric embryo images.

Dr. Obokata explained that she obtained the two images from Dr. Wakayama, and with Dr. Sasai used them in preparing the figures for the paper. During the preliminary production of the paper, Dr. Obokata inserted the image under Fig. 2g as a control for comparison between STAP cells and the FI stem cells. Then, in the process of writing by Dr. Sasai, the structure of the paper changed, the order of the figures changed, and the image became unnecessary, so a decision was made not to include it. However, Dr. Obokata explained that they forgot to remove the image when they were editing the figures for the paper. Dr. Sasai further explained that the paper was submitted without him realizing the image had not been deleted, and that he failed to notice this during the editing and proofreading processes. He also explained that he neglected to instruct Dr. Obokata to delete the figure.

The bottom image of Figure 2g shows placenta GFP expression, but both the text and caption refer to embryo GFP expression, which only explains the top image of Figure 2g. The investigative committee was presented with date-stamped files showing the original structure of the figures with the location of the images and copies of the corresponding lab notes.

Opinions

The fluorescence placenta in Figure 1b (right panel) and that in Fig. 2g (bottom panel) are images that originated from the same chimera. There are, however, other images in the paper that are not referred to, either in the text or the figure legends, and it is possible to surmise that the bottom panel of Fig. 2g was included to show the existience of GFP-positive cells. Still, considering the fact that not all versions of the paper's editing were preserved so that the investigative committee could reconstruct the exact process as had been explained, it is plausible, given the date-stamped file data described above, that there was a previous version with the figures in a different position. As has already been pointed out, there are other images in the paper that are not referred to, either in the text or figure legends. Further investigation suggests that while there may be other reasons for these omissions besides forgetfullness, there are no materials that directly indicate anything exceeding negligence . Although this could be considered "falsification" as defined in RIKEN's Regulations on the Prevention of Research Misconduct, there is no evidence suggesting anything exceeding negligence, and this, therefore, is not judged to constitute research misconduct.

3. Summary

We concluded that there was research misconduct by Dr. Obokata on two points. Research misconduct warps the essence of science and significantly undermines credibility, not only within the science community, but also with the general public. Research misconduct is prohibited precisely because of the need to ensure robust, healthy exchange of information among scientists in their search for truth, and to promote the advancement of science. In manipulating the image data of two different gels and using data from two different experiments, Dr. Obokata acted in a manner that can by no means be permitted. This cannot be explained solely by her immaturity as a researcher. Given the poor quality of her laboratory notes it has become clearly evident that it will be extremely difficult for anyone else to accurately trace or understand her experiments, and this, too, is considered a serious obstacle to healthy information exchange. Dr. Obokata's actions and sloppy data management lead us to the conclusion that she sorely lacks, not only a sense of research ethics, but also integrity and humility as a scientific researcher. We were also forced to conclude that the normal system by which senior researchers should have been carefully checking all raw data did not work in this case. None of the other persons investigated were found to have actively participated in any kind of research misconduct, but as has already been noted, Drs. Wakayama and Sasai allowed the papers to be submitted to Nature without verifying the accuracy of the data, and they bear heavy responsibility for the research misconduct that resulted from this failure on their part.

Among the possible reasons for the failure of the normal system of checking on research results, is the change in research environment and the involvement of several senior researchers. Dr. Obokata continued research she had started at another institution at CDB, first while working in the Wakayama laboratory as a visiting researcher, and then later as the head of her own laboratory. As she neared the point of attaining results, Drs. Sasai and Niwa, two senior researchers other than Dr. Wakayama, became involved in reinforcing the data and writing the papers.

RIKEN must examine why the normal checking mechanisms did not function as they should have, and reconsider such issues as how responsibility should be allocated among different groups working together on joint research and among paper co-authors. RIKEN should also reexamine the whole process of data management, including the

management of laboratory notebooks, as well as the process from research proposal to collating results and presenting them in papers. RIKEN must promptly institute specific measures to ensure that this kind of research misconduct will never happen again.

RIKEN Research Paper Investigative Committee

Chair	
Ishii, Shunsuke	Distinguished Senior Scientist, RIKEN Molecular Genetics Laboratory
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Watanabe, Jun	Attorney at Law, Watanabe Law Office