



RESEARCH ARTICLE

Transient acid treatment cannot induce neonatal somatic cells to become pluripotent stem cells [v1; ref status: indexed, <http://f1000r.es/3dqj>]

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v1 First published: 08 May 2014, 3:102 (doi: [10.12688/f1000research.4092.1](https://doi.org/10.12688/f1000research.4092.1))
Latest published: 08 May 2014, 3:102 (doi: [10.12688/f1000research.4092.1](https://doi.org/10.12688/f1000research.4092.1))

Abstract

Currently, there are genetic- and chemical-based methods for producing pluripotent stem cells from somatic cells, but all of them are extremely inefficient. However, a simple and efficient technique has recently been reported by Obokata *et al* (2014a, b) that creates pluripotent stem cells through acid-based treatment of somatic cells. These cells were named stimulus-triggered acquisition of pluripotency (STAP) stem cells. This would be a major game changer in regenerative medicine if the results could be independently replicated. Hence, we isolated CD45⁺ splenocytes from five-day-old Oct4-GFP mice and treated the cells with acidified (pH 5.7) Hank's Balanced Salt Solution (HBSS) for 25 min, using the methods described by Obokata *et al* 2014c. However, we found that this method did not induce the splenocytes to express the stem cell marker Oct4-GFP when observed under a confocal microscope three to six days after acid treatment. qPCR analysis also confirmed that acid treatment did not induce the splenocytes to express the stemness markers *Oct4*, *Sox2* and *Nanog*. In addition, we obtained similar results from acid-treated Oct4-GFP lung fibroblasts. In summary, we have not been able to produce STAP stem cells from neonatal splenocytes or lung fibroblasts using the acid-based treatment reported by Obokata *et al* (2014a, b, c).

Article Status Summary

Referee Responses

Referees	1	2
v1 published 08 May 2014	 report 1	 report 1

1 **Christine Mummery**, Leiden University
Medical Center Netherlands

2 **Janet Rossant**, Hospital for Sick Children
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Latest Comments

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12 May 2014 (V1)

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10 May 2014 (V1)

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How to cite this article: Tang MK, Lo LM, Shi WT *et al.* (2014) **Transient acid treatment cannot induce neonatal somatic cells to become pluripotent stem cells [v1; ref status: indexed, <http://f1000r.es/3dqj>]** *F1000Research* 2014, **3**:102 (doi: [10.12688/f1000research.4092.1](https://doi.org/10.12688/f1000research.4092.1))

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Grant information: This research has been supported by the Hong Kong Cancer Fund (CUHK project number 6903321) assigned to Professor Kenneth Lee.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: No competing interests were disclosed.

First published: 08 May 2014, **3**:102 (doi: [10.12688/f1000research.4092.1](https://doi.org/10.12688/f1000research.4092.1))

First indexed: 21 May 2014, **3**:102 (doi: [10.12688/f1000research.4092.1](https://doi.org/10.12688/f1000research.4092.1))

Introduction

Takahashi and Yamanaka (2006) reported that it was possible to induce adult fibroblasts into pluripotent stem cells using four factors: Oct3/4, Sox2, c-Myc and Klf4. The creation of these induced pluripotent stem (iPS) cells and their replication in human somatic cells has been heralded as a major breakthrough in regenerative medicine (Takahashi *et al.*, 2007). However, the method for generating iPS cells involves complex genetic manipulation and it is beset by low efficiency of conversion and yield (Liao *et al.*, 2008). Currently, great strides are being made to improve the efficiency of iPS cell production by incorporating small molecules, vitamin C, valproic acid, microRNAs and different combinations of transcriptional factors into the protocols (reviewed by Huo & Zambidis, 2013; Jung *et al.*, 2014). Two recent sets of groundbreaking studies reported in *Nature* appear to overcome most of these problems (Obokata *et al.*, 2014a; Obokata *et al.*, 2014b). The authors reported that simply bathing somatic cells in a mild acid could reprogram them to become pluripotent stem cells. They harvested spleen cells from 1-week-old Oct4-GFP transgenic mice, and isolated the CD45⁺ population by flow cytometry. The CD45⁺ splenocytes were then treated with acidified HBSS (pH 5.7) for 25 min at 37°C and maintained in DMEM/F-12 culture medium containing B27 and Leukemia Inhibitory Factor (LIF) for one to seven days. This simple procedure activated the Oct4 promoter two days post-treatment, making the splenocytes express the GFP reporter.

The authors named the cells generated by this procedure “Stimulus-triggered acquisition of pluripotency (STAP) stem cells” (Obokata *et al.*, 2014a). STAP cells were demonstrated to be able to form all organs and tissues in chimeric embryos, when injected into host blastocysts (Obokata *et al.*, 2014b). Furthermore, the authors reported that STAP cells could also contribute to the placenta, something that iPS and ES cells are normally incapable of doing (Rossant, 2008). In other words, STAP cells appear to be totipotent rather than pluripotent. If these surprising findings could be confirmed, it would revolutionize regenerative medicine by providing a simple, cheap, and immunocompatible source of stem cells for tissue/organ repair.

There are several fundamental questions that need to be addressed before STAP stem cells can be accepted as one of the main methods for generating stem-like iPS cells, such as: Can these findings be replicated by other researchers? And, other than mouse spleen cells, can this STAP protocol be applied to other somatic cells? Since the publication of the STAP articles, there has been a recent groundswell of comments on social media platforms, including blogs (Knoeffler Lab Stem Cell Blog), networking sites (ResearchGate) and Twitter, that have cast considerable doubt on Obokata *et al.*'s findings. In this context, we have attempted to create STAP cells using their most updated protocol (Obokata *et al.*, 2014c).

Materials and methods

Animals

Oct4-GFP transgenic (CBA-Tg (Pou5f1-EGFP) 2Mnn/j) mice, obtained from The Jackson Laboratory and maintained in the CUHK Laboratory Animal Services Centre, were used for experimentation. The usage of these mice was approved by the CUHK Animal Experimentation Ethics Committee (Project No.: 11/056/GRF-5).

The new born neonates were kept in a nest bedding with their mothers at 25°C room temperature, under a 12/12 dark-light cycle, and sufficient food and water. We humanely euthanized six to seven of five-day-old neonatal Oct4-GFP mice by cervical dislocation (according to the ARRIVE guidelines, Kilkenny *et al.*, 2012) and harvested their testes, spleens and lungs. This was done on the same day as the mice left animal breeding centre.

Germ cell isolation and analysis

Testes were harvested from five-day-old neonates and maintained in HBSS medium. The seminiferous tubules were immediately isolated from the testes, under a Nikon SMZ745T stereo dissecting microscope. The tubules were then kept, and maintained in DMEM medium supplemented with 10% qualified Fetal bovine serum (FBS) (Gibco®, Invitrogen; Cat#10270) and penicillin (100U/mL)-streptomycin (0.1mg/mL) (Gibco®, Invitrogen; Cat#15140122). The tubules were examined under a confocal microscope to determine whether the germ cells could express Oct4-GFP. Single germ cell suspensions were also produced from the seminiferous tubules using methods described by (Garcia & Hofmann, 2012). These germ cells were analyzed using a BD LSRFortessa™ Cell Analyzer (BD Biosciences, USA) to determine whether these cells were capable of expressing Oct4-GFP.

Preparation of splenocytes and lung fibroblasts from neonatal mice

Spleens were isolated from five-day-old neonates capable of expressing Oct4-GFP when appropriately induced (e.g. using OSMK factors). The spleens were first mechanically passed through a cell strainer (grid size 70µm) to disperse the tissues and dissociate the splenocytes. The splenocytes were pelleted by centrifugation at 1200 rpm for five min and resuspended in ACK lysis buffer (65mM NH₄Cl, 10mM KHCO₃ and 0.1M Na₂-EDTA in distilled H₂O, adjusted to pH 7.3) for five min at room temperature to remove residual erythrocytes. The splenocytes were then resuspended in DMEM supplemented with 10% FBS, and 1% PS. The crude splenocytes were maintained at 37°C and 5% CO₂ for one to six days.

Besides splenocytes, we also isolated fibroblasts from the lungs of the Oct4-GFP neonates (Yau *et al.*, 2011). Briefly, explants were prepared from dissected pieces of the lung (approximately 1 mm² in size). The explants were treated with 1 mg/ml collagenase Type I (Gibco®, Invitrogen) for 30 min. The explants were then washed with PBS and plated onto collagen-coated 100 mm culture dishes (SPL; Cat#20101). DMEM/F12 plus 10% FBS were added to the explants (just enough to cover the explants). These explants were then maintained at 37°C and 5% CO₂, with the culture medium changed every three days. Fibroblasts could be observed migrating out of the explants after two days. After seven days, the explants were removed and the fibroblasts on the culture dishes were trypsinized with 0.25% Trypsin-EDTA (Gibco®, Invitrogen) for two minutes and sub-cultured.

Isolation of CD45⁺ splenocytes

The crude splenocytes produced above were resuspended in PBS into a single-cell (1×10⁷ cells/ml) suspension. The cells were incubated with Anti-mouse CD45 antibodies, which were directly conjugated

with FITC (1: 100 dilutions, BD Pharmingen™ FITC Rat Anti-mouse CD45) for 30 min at 4°C. The stained cells were then washed three times with PBS and resuspended in PBS supplemented with 1% FBS. The CD45⁺-stained splenocytes were sorted and purified using a Cell Sorter (BD LSRFortessa Cell Analyzer). The cells were sorting on 3 different occasions using the same pool of splenocytes extracted from the spleens of 6 neonates.

Acid treatment of CD45⁺ splenocytes

We treated the somatic cells with low-pH medium according to the protocol described in (Obokata *et al.*, 2014c). Accordingly, 5×10⁵ cells/ml of CD45⁺ splenocytes or lung fibroblasts were treated with low-pH HBSS (adjusted to pH 5.7 with HCl) for 25 min at 37°C. The acid-treated cells were then centrifuged at 1200 rpm for 5 min. The supernatants were removed and rechecked to confirm that the pH was still pH 5.7. All pH were measured using a calibrated pH meter (Mettler-Toledo, USA). All of acid-treated cell pellets were resuspended in DMEM/F-12 medium supplemented with 1× B27 and 1,000U LIF at a concentration of 1×10⁵ cells/ml. The cultures were then plated onto non-adhesive culture dishes (SPL; Cat#11035) and examined for GFP expression on seven consecutive days using microscopy (LEICA SP5). The experiments were performed in triplicate.

Real-Time quantitative PCR analysis

The acid-treated and untreated cells were harvested for qPCR after six-seven days of culture. Briefly, total RNAs were isolated using an RNeasy® mini kit (Qiagen, USA) and reverse transcribed using an Omniscript RT Kit (Qiagen, USA). We used National Institutes of Health's qPrimer Depot Database to determine the primers for *Oct4* (forward: 5'GTTGGAGAAGGTGGAACCAA3'; reverse: 5'TCTTCTGCTTCAGCA GCTTG3'), *Sox2* (Forward: 5'ACAA-GAGAATTGGGAGGGGT3'; reverse: 5'AAAGCGTTAATTTG-

GATGGG3') and *Nanog* (forward: 5'CCAGTGGAGT ATCCCAGCAT3'; reverse: 5'GAAGTTATGGAGCGGAG CAG3') expressions. The master mix for each qPCR sample was prepared to a total volume of 5 µl for a 384 well-plate: 10 ng cDNA template, 0.55 µM forward primer, 0.55 µM reverse primer, SYBR green master premix (Takara Biotechnology Co Ltd, Dalian) and RNase-free water (Ambion, Cat#AM9937). The qPCR was performed with a denaturation step at 95°C for 30 sec and thermal profiling (denaturation step: 95°C, 5 sec; annealing and extension steps: 42°C, 30 sec) for 40 cycles (ABI ViiA 7 Real Time PCR System). After the process was completed, the dissociation and amplification curves were checked to see if there was any abnormal amplification. The Ct values were further measured and acquired. The data were generated for quantitative analyses after normalization with GAPDH housekeeping gene. All samples were run in triplicate.

Statistics

The data were analyzed using two-tailed, paired student's t-test. P<0.05 was considered to be statistically significant. The statistical analysis was performed using SPSS software version 22.

Results

Validation of Oct4-GFP transgenic mice

We first confirmed that the cells in our Oct4-GFP transgenic mice were capable of expressing Oct4-GFP. It has been reported that all of the spermatogonia in the testes of this type of transgenic mice were capable of expressing Oct4 (Denn *et al.*, 2008). Hence, we isolated the seminiferous tubules from the testes of our transgenic mice and directly examined the tubules under a confocal microscope. We determined that there were numerous Oct4-GFP⁺ spermatogonia present in the tubules (Figure 1A). This was further validated by flow cytometry of dissociated germ cells extracted from the seminiferous tubules (Figure 1B).

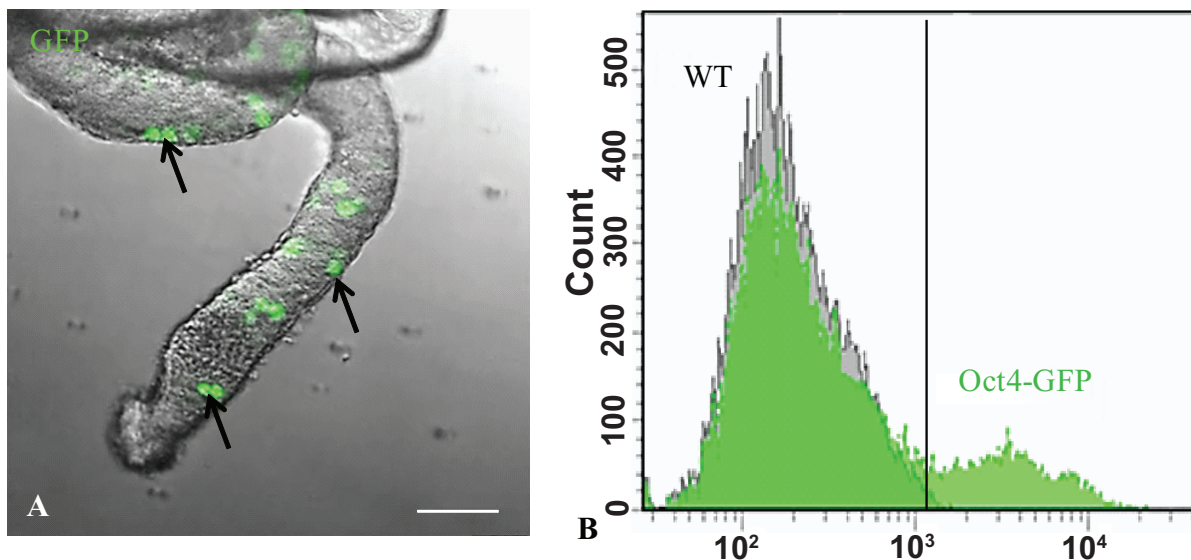


Figure 1. Analysis of germ cells from the testes of five-day-old Oct4-GFP neonates. (A) Confocal image of seminiferous tubules isolated from five-day-old neonates showing the germ cells expressing Oct4-GFP (arrows). Bar = 50µm. (B) Flow cytometry histogram confirming that the germ cells are capable of expressing the transgene. The peaks show that only Oct4-GFP germ cells but not wild type (WT) germ cells expressed GFP.

Effects of acid treatment on the neonatal Oct4-GFP splenocytes and lung fibroblasts

To replicate Obokata's experiment, we isolated a pure population of CD45⁺ splenocytes from the spleens of five-day-old Oct4-GFP mice as shown in **Figure 2A**. The CD45⁺ splenocytes were treated with a mild acidic (pH 5.7) HBSS for 25 min at 37°C. The acid-bathed cells were then cultured in DMEM/F-12 medium supplemented with B27 and 1,000U LIF. We examined the cells for Oct4-GFP expression, every day for up to seven days, but did not observe any Oct4-GFP expression under the confocal microscope (**Figures 2B, C and D**). However, we did occasionally observe clusters of cells that appeared to be GFP⁺ but were later determined to be autofluorescence, as these cells were necrotic and stained positive

with propidium iodide (**Figure 2E**). qPCR analysis was performed to establish whether the acid-bathed cells were capable of expressing the stemness markers. The qPCR results revealed that acid treatment did not induce the splenocytes to express *Oct4*, *Sox2* and *Nanog* (**Figure 2F**).

We also attempted to produce STAP cells from Oct4-GFP lung fibroblasts. Like the splenocytes, the fibroblasts were treated with acidic HBSS (pH 5.7) for 25 min at 37°C. We checked the cells for GFP expression for seven consecutive days but did not observe any GFP expression (**Figure 3A and B**). Likewise, our qPCR analysis also did not show induced *Oct4*, *Sox2* and *Nanog* expressions in our acid-treated fibroblasts (**Figure 3C**).

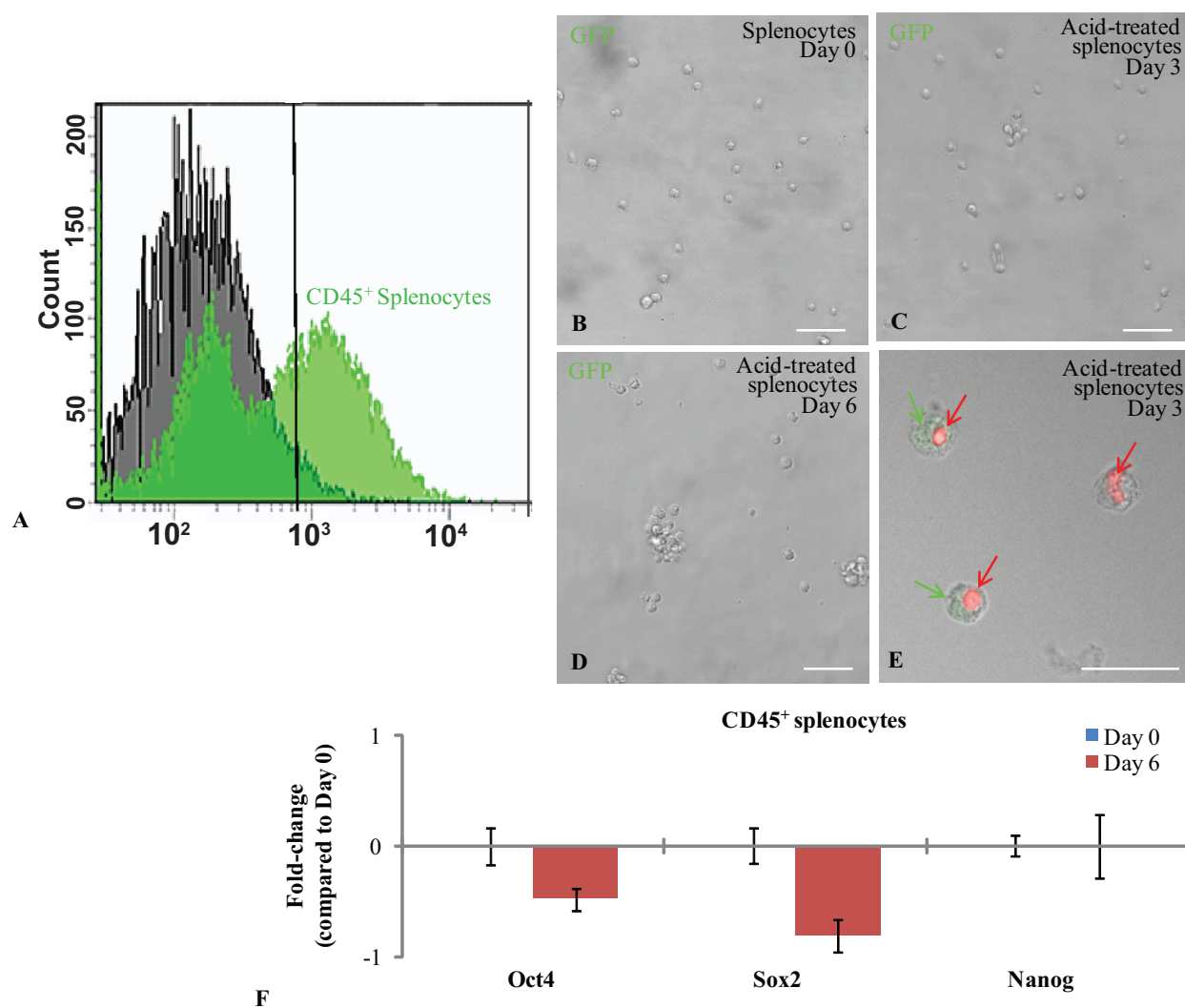


Figure 2. Induction of Oct4-GFP expression in acid-treated splenocytes. (A) Flow cytometry histogram showing the sorted CD45⁺ splenocytes used in our experiments. (B–E) Confocal images of acid-treated splenocytes after zero to six days of culture. No Oct4-GFP expression was detected in the splenocytes at any of the time-points analyzed. Bar = 50µm. (E) Confocal image of acid-treated splenocytes stained with Propidium iodide (PI) after three days of culture. The PI dye identified the necrotic splenocytes (red arrows) which occasionally also emitted a green autofluorescence (green arrows). Bar = 25µm. (F) qPCR analysis also confirmed that acid-treatment did not induce the splenocytes to express the stemness markers *Oct4*, *Sox2* and *Nanog*. Error bars represent standard error of the mean (p ≤ 0.05).

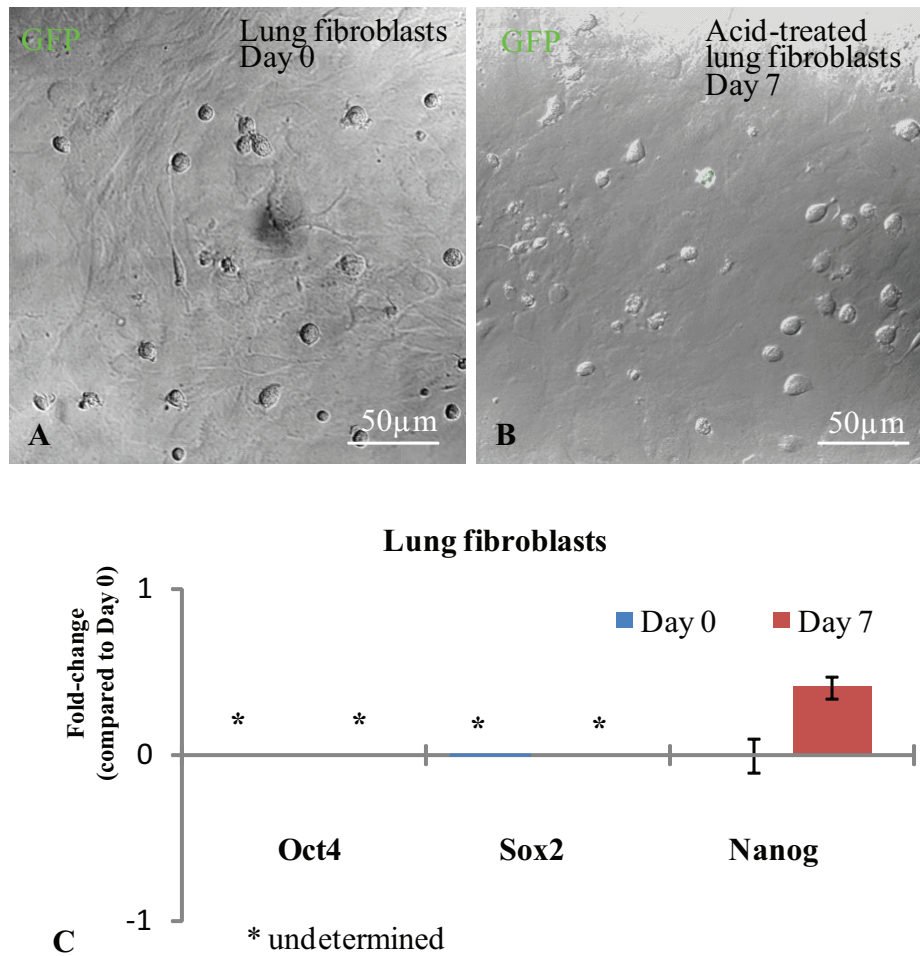


Figure 3. Induction of Oct4-GFP expression in acid-treated lung fibroblasts. (A and B) Confocal images of acid-treated fibroblasts zero and seven days after culture. None of the acid-treated fibroblasts expressed the Oct4-GFP transgene. (C) qPCR analysis demonstrated that acid-treatment did not induce *Oct4*, *Sox2* and *Nanog* expression. No expression was determined after 40 cycles of qPCR for *Oct4* and *Sox2*, hence marked as 'undetermined'. Error bars represent standard error of the mean ($p \leq 0.05$).

Dataset 1 and 2. qPCR results of CD45⁺ splenocytes/lung fibroblasts

2 Data Files

<http://dx.doi.org/10.6084/m9.figshare.1014318>

Discussion

Currently, there is a trend to simplify iPS cell production by minimizing genetic manipulation and incorporating the use of small chemical molecules for somatic cell reprogramming (Shi *et al.*, 2008; Zhu *et al.*, 2010). In this context, it has been reported that mouse iPS cells could be generated using a cocktail of seven chemical molecules without any genetic manipulation, with an efficiency of around 0.2% (Hou *et al.*, 2013). These important developments were recently superseded by claims that hydrochloric acid treatment alone can chemically reprogram fibroblasts to become induced pluripotent stem cells (Obokata *et al.*, 2014a; Obokata *et al.*, 2014b). The authors claimed that chemically stressing CD45⁺ splenocytes (isolated from Oct4-GFP neonates) to the point of death so that

approximately 25% of the cells survive will activate the Oct4-GFP transgene in over 50% of surviving cells. They called these cells "STAP cells" which, when injected into host blastocysts, could participate in the development of all tissues and organs, including the placenta. The STAP chimeric mice produced were reported to be healthy, and the STAP-derived germ cells were demonstrated to be involved in germline transmission (Obokata *et al.*, 2014a; Obokata *et al.*, 2014b). These are astonishing findings. Nevertheless, we have tried to replicate the first stages of Obokata's findings using CD45⁺ splenocytes isolated from Oct4-GFP neonates, but could not activate the expression of the Oct4-GFP transgene. This is despite using their most updated protocol for producing STAP cells, which was reported in *Protocol Exchange* (Obokata *et al.*, 2014c). We also tried using Oct4-GFP lung fibroblasts instead of splenocytes, but again we failed to detect Oct4-GFP expression after acid-treatment. Occasionally, there were cells that appeared GFP positive, but we later determined them to be autofluorescence from apoptotic cells. We made sure that the pH was exactly maintained at pH 5.7 during the experiments by measuring the pH before and after cell treatment. This is because the *Protocol Exchange* protocol placed a lot

of emphasis on maintaining an optimal pH during the acid treatment of the cells. We found that there was a pH 0.1 increase after the acid buffer was added to treat the cells – so our starting pH was actually 5.6 to compensate. At the end of acid bath stimulation, we also measured the pH of the buffer to confirm that it was still pH 5.7. Therefore, our inability to produce STAP cells could not be attributed to changes in the pH during the cell stimulus procedures.

Another possibility why we could not replicate Obokata's results might be the difference in the strains of Oct4-GFP transgenic mice used. We acquired our transgenic mice from The Jackson Laboratory (CBA-Tg (Pou5f1-EGFP) 2Mnn/j) while Obokata used transgenic mice generated by Ohbo *et al.*, 2003. Their transgenic mice were developed from a C57BL.6J background, and carry the EGFP cDNA under the control of an Oct4 18-kb genomic fragment (consisting of a minimal promoter and proximal and distal enhancer). Perhaps the transgene in these mice is more easily activated than in our Jackson Laboratory mice. This could potentially explain why Obokata's transgenic splenocytes, but not our transgenic splenocytes, expressed the EGFP reporter following acid bath treatment. Nevertheless, in the context of generating STAP stem cells, it is not the expression of the transgene that is important but rather the expression of the endogenous Oct4 gene - and related endogenous stemness genes, Sox2 and Nanog. Expression of these genes could not be demonstrated using qPCR analysis following splenocyte acid treatment and culture.

In conclusion, we have not been able to replicate Obokata *et al.*'s findings to produce STAP stem cells from somatic cells. It appears that the method for producing STAP stem cells is not as simple and straight forward as has been reported.

Data availability

figshare: Dataset 1 and 2. qPCR results of CD45+ splenocytes/lung fibroblasts. Doi: [10.6084/m9.figshare.1014318](https://doi.org/10.6084/m9.figshare.1014318) (Tang *et al.*, 2014)

Author contributions

Mei Kuen Tang performed the confocal microscopy experiments, analysed the data and was overall in charge of the experiments performed in this study.

Lok Man Lo performed the experiments of the transgenic splenocytes.

Wen Ting Shi performed the experiments on the transgenic lung fibroblasts.

Yao Yao isolated the tissues from the transgenic mice and performed the flow cytometry experiments.

Henry Siu Sum Lee assisted in the production of the manuscript and figures.

Kenneth Ka Ho Lee wrote the manuscript.

Competing interests

No competing interests were disclosed.

Grant information

This research has been supported by the Hong Kong Cancer Fund (CUHK project number 6903321) assigned to Professor Kenneth Lee.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Tang MK, Lo LM, Shi WT, *et al.*: **Dataset 1 and 2. qPCR results of CD45+ splenocytes/ lung fibroblasts.** *figshare*. 2014.

[Data Source](#)

Yau WW, Tang MK, Chen E, *et al.*: **Cardiogenol C can induce Mouse Hair Bulge Progenitor Cells to Transdifferentiate into Cardiomyocyte-like Cells.** *Proteome*

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[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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Current Referee Status:

Referee Responses for Version 1



Janet Rossant

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Approved: 21 May 2014

Referee Report: 21 May 2014

doi:[10.5256/f1000research.4382.r4845](https://doi.org/10.5256/f1000research.4382.r4845)

Tang *et al.* report attempts to replicate the findings of *Obokata et al.* on the derivation of pluripotent STAP cells by acid treatment of mouse splenocytes. Despite attempting to replicate the exact details of the Obokata experiments as published, they were not able to observe any activation of an Oct4-GFP transgene or of the endogenous pluripotent markers after several days of culture of the treated cells.

While these results do suggest that the STAP protocol is not simple and easily replicated, negative findings are always hard to interpret and need to be very carefully controlled. For this reason there are some points that need to be considered:

1. It is not clear how much cell death occurred after the acid treatment - was this at the same level as reported by *Obokata et al.*?
2. What was the sensitivity of the PCR analysis of the expression of the pluripotent genes? A control analysis of different levels of expression in existing pluripotent lines would help.
3. Is it possible to repeat the analysis with a different Oct4-reporter to avoid the potential confusion with green autofluorescence?

There is merit in this replication study being published in the scientific literature, however when trying to reproduce a null finding the controls used need to be well defined to prevent any ambiguity.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

1 Comment

Author Response

Kenneth Lee, School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong
Posted: 24 May 2014

Professor Rossant has made some excellent suggestions for improving the paper.

Points 2 and 3 will be address by adding positive and negative controls into a revised version of the paper. This will demonstrate the sensitively of our Oct4-GFP transgene. qPCR data will also

accompany these results which will address the sensitivity of the PCR analysis question. To address point 1, how “much cell death occurred after the acid treatment”, we are currently checking over all our stored confocal images that have been stained with PI dye to derive an estimate. We will try and integrate this data into the revised version of the paper.

To date, we have already generated a set of negative control results but the positive control will take some time to generate. We will do it by chemical induction of our Oct4-GFP lung fibroblasts using a cocktail of small molecules. We will try and have a revised version of the paper uploaded as soon as possible.

Competing Interests: I am the corresponding author of the paper.



Christine Mummery

Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands

Approved: 09 May 2014

Referee Report: 09 May 2014

doi:[10.5256/f1000research.4382.r4727](https://doi.org/10.5256/f1000research.4382.r4727)

The authors describe their attempt to reproduce a study in which it was claimed that mild acid treatment was sufficient to reprogramme postnatal splenocytes from a mouse expressing GFP in the oct4 locus to pluripotent stem cells. The authors followed a protocol that has recently become available as a technical update of the original publication.

They report obtaining no pluripotent stem cells expressing GFP driven over the same time period of several days described in the original publication. They describe observation of some green fluorescence that they attributed to autofluorescence rather than GFP since it coincided with PI positive dead cells. They confirmed the absence of oct4 expression by RT-PCR and also found no evidence for Nanog or Sox2, also markers of pluripotent stem cells.

The paper appears to be an authentic attempt to reproduce the original study, although the study might have had additional value with more controls: “failure to reproduce” studies need to be particularly well controlled.

Examples that could have been valuable to include are:

1. For the claim of autofluorescence: the emission spectrum of the samples would likely have shown a broad spectrum not coincident with that of GFP.
2. The reprogramming efficiency of postnatal mouse splenocytes using more conventional methods in the hands of the authors would have been useful as a comparison. Idem the lung fibroblasts.
3. There are no positive control samples (conventional mESC or miPSC) in the qPCR experiments for pluripotency markers. This would have indicated the biological sensitivity of the assay.
4. Although perhaps a sensitive issue, it might have been helpful if the authors had been able to obtain samples of cells (or their mRNA) from the original authors for simultaneous analysis.

In summary, this is a useful study as it is citable and confirms previous blog reports, but it could have been improved by more controls.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

1 Comment

Author Response

Kenneth Lee, School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong

Posted: 12 May 2014

Professor Mummery has provided some excellent suggestions for changes to improve the paper. We will try our best and accommodate her requests 1-3 by doing some new additional experiments.

Request 4 will be next to impossible to address, I don't think Haruko Obakata will be willing to give us her STAP stem cells- even if she does it will be problematic for us to establish they were originally derived from mouse splenocytes.

Competing Interests: I am the corresponding author of this paper.

Article Comments

Comments for Version 1

Author Response

Kenneth Lee, School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong

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Pipetting and then acid-treating Oct4-GFP fibroblasts using Vancanti's protocol **did not induce** the Oct4-GFP fibroblasts to express endogenous Oct4, Sox2 and Nanog, as assessed by qPCR (ResearchGate Blog:

https://www.researchgate.net/publication/259984904_Stimulus-triggered_fate_conversion_of_somatic_cells_into_pluripotency/reviews/103.).

However, pipetting Oct4-GFP fibroblasts alone (**the negative control**) did induce the Oct4-GFP fibroblasts to express endogenous Oct4, Sox2 and Nanog in the first blogged experiment. The Oct4 and Nanog expressions were increased by approximately 8 folds and Sox2 by one fold. This **level is far too low** - as conventional iPS reprogramming induces the expression of these 3 genes in somatic cells by hundreds of folds (**See Figure 3 of the ResearchGate Blog**).

Moreover, in the second series of Vancanti's fibroblasts pipetting alone experiment (negative control), the qPCR results did not show any meaningful increase in Oct4, Sox2 nor Nanog expression (**see the last**

Bar Chart of the ResearchGate Blog). It appears that dying fibroblasts (because of the stress induced by pipetting) can occasionally and randomly flicker on these stemness genes. [**Note: no acid treatment was involved in these two series experiments**].

Claims that we repeated Vancanti's STAP stem cell experiment were **hyped up by the press**. Closer examination of the findings in our Blog clearly show that it is untrue.

Competing Interests: Corresponding author of the paper

Charles Lund, MIT, USA

Posted: 10 May 2014

A few weeks ago, the same group claimed that they could repeat, at least partially, the STAP cells using the protocol published from Dr. Vacanti's group.

Competing Interests: no competing interests
