

Research Assessment Exercise 2020
Impact Case Study

University: The Chinese University of Hong Kong

Unit of Assessment (UoA): 02 (Pre-clinical studies)

Title of case study: Serendipity consequence of debunking the existence of STAP stem cells

(1) Summary of the impact (indicative maximum 100 words)

Peer review is critical in science development and progression. In 2014, Nature reported that fibroblasts could be reprogrammed into pluripotent STAP stem cell by exposure to acid. (i) Lee was the first to attempt replicating this revolutionary finding but failed. Despite initial scepticism from Nature's editors, his findings were subsequently confirmed by seven leading stem cell laboratories. Harnessing social media and open peer-review journal transformed the impact in the stem cell field; (ii) inspired a US patent and revenue generating start-up biotech company; (iii) a block-buster movie; and (iv) a forerunner in the use of social media for scientific communications.

(2) Underpinning research (indicative maximum 500 words)

Underpinning research was undertaken at CUHK, led by Prof. Kenneth Lee (at CUHK 2007-2019).

Chemical-based methods for reprogramming somatic cells into stem-like cells

Apart from gene-based methods in reprogramming somatic cells, scientists have long known to use small molecules to induce differentiated cells to become stem cells. Lee delineated how a small molecule, Reversine, can reprogram C2C12 myogenic cells to various differentiated cell types. He also investigated another small molecule, Cardiogenol C, in inducing mouse hair bulge progenitor cells to transdifferentiate into cardiomyocyte-like cells (**Ref. 3.1**).

Gene-based studies to understand the molecular control of stem cell self-renewal

During early development and throughout the lifespan of mammalian species, a balance between stem cell self-renewal and differentiation into specialized cell types is critical for organismic survival. Dysregulation can result in developmental defects or cancer. Lee studied the *BRE* gene in stem cell biology. BRE is a multifunctional adaptor protein involved in DNA repair, cell survival and stress response. Using human umbilical cord perivascular progenitor cells, Lee determined the ability of BRE in promoting stem cell renewal through upregulating a host of stem cell genes *OCT4*, *FGF5* and *FOXO1A* (**Ref. 3.2**). Using CRISPR/Cas9 techniques, Lee also demonstrated the importance of Bag1 in the maintenance of stemness in embryonic stem cells (**Ref. 3.3**).

Studies of Stimulus-Triggered Acquisition of Pluripotency (STAP) cells

Reprogramming of somatic cells to acquire pluripotency was pioneered by J. Gurdon in an amphibian model and subsequently by S. Yamanaka by introducing four transcription factor genes into mature cells. These seminal findings were awarded the Nobel Prize in Physiology or Medicine in 2012. In 2014, Obokata et. al. reported in *Nature* that similar reprogramming was achieved by simply exposing cells to low pH, referring to this procedure as “stimulus-triggered acquisition of pluripotency” or STAP. This work received considerable attention because of the revolutionary way in which stem cells could be generated through a simple and low-cost protocol. Lee was first to attempt reproducing Obokata's work. An Oct4-GFP mouse strain was used as a reporter system to monitor the stemness of somatic cells. In this model, the GFP expression was under the control of the promoter/enhancer elements of the stem cell marker, Oct4. Thus, “stemness” could be monitored by the fluorescent signal generated from the GFP protein. However, the treatment of Oct4-GFP somatic cells with acidified (pH 5.7) culture medium did not result in the generation of any fluorescent signal. In fact, none of the other stem cell markers such as *Sox2* and *Nanog* became upregulated. Concluded STAP cell do not

exist (**Ref. 3.4**). However, Lee serendipitously discovered that mouse and human fibroblasts cultured as spheroids can be induced to express low levels of pluripotent-associated gene *Oct4*, *Sox2* and *Nanog* (**Ref. 3.5**).

(3) References to the research (indicative maximum of six references)

(*Corresponding author)

- 3.1 Induction of growth arrest and polycomb gene expression by reversine allows C2C12 cells to be reprogrammed to various differentiated cell types. (2007) Shan SW, Tang MK, Chow PH, Morato M, Cai DQ, Lee KKH*. *Proteomics* 7(23):4303-4316. DOI:10.1002/pmic.200700636.
- 3.2 Silencing BRE expression in Human Umbilical Cord Perivascular (*HUCPV*) progenitor cells accelerates osteogenic and chondrogenic differentiation. (2013) Chen E, Tang MK, Yao Y, Yau WWY, Lo LM, Yang X, Chui YL, Chan JYH, Lee KKH*. *PLoS ONE* 8(7):e67896. DOI:10.1371/journal.pone.0067896.
- 3.3 Generation of a Bag1 homozygous knockout mouse embryonic stem cell line using CRISPR/Cas9. (2017) Tang CC, Shan LP, Wang WM, Lu G, Tare RS, Lee KKH*. *Stem Cell Research* 21:29-31. DOI: 10.1016/j.scr.2017.03.016.
- 3.4 Transient acid treatment cannot induce neonatal somatic cells to become pluripotent stem cells. (2014) Tang MK, Lo LM, Shi WT, Yao Y, Lee HSS, Lee KKH*. *F1000Research* 3:102. DOI: 10.12688/f1000research.4382.
- 3.5 Growing human dermal fibroblasts as spheroids renders them susceptible for early expression of pluripotency genes (2019). Lo LM, Raghunath M, Lee KKH*. *Advanced Biosystems* 3(10), 1900094. DOI:10.1002/adbi.201900094.

(4) Details of the impact (indicative maximum 750 words)

Reprogramming somatic cells to acquire pluripotency was a Nobel Prize winning discovery. If true, STAP could have had a world-wide impact. Lee was the first to try replicating the now highly controversial “STAP Stem cells” experiment using real-time experimentation on social media ResearchGate and Twitter. This real-time experiment created a great deal of interest from mainstream media including BBC Health, Wall Street Journal, The Economist, Nature (news), Science Magazine (news), WIRED (UK and Germany), The Scientist, The Guardian, and NHS Health. Fuji Television Network filmed and broadcasted Lee’s STAP Stem Cell replication efforts in Japan (**Ref. 5:10**). The program was watched by millions of Japanese because of their intense interest in the STAP scandal and Lee was the only laboratory that provided a scientific insight. On day1 after the program, there were 263 mainly positive comments on the internet and generated over 1,000 thread. Millions of Japanese viewers also learnt about Lee’s STAP study on NHK News broadcasted the same day.

The ResearchGate website where the real-time STAP experiment was conducted had received 25,000 hits per day over a period of 5 days. Lee's research team at the end of experimentation reported that it was not possible to produce STAP stem cells and that the phenomenon did not exist despite “STAP cells” being reported in two highly publicized Nature articles. Lee submitted his findings to *Nature* but the article was rejected amidst an outcry of skepticism and criticism from the scientific community. However, the article was soon identified by two leading stem cell experts (C. Mummery and J. Rossant) as deserving reassessment, resulting in an open peer review paper appearing in *F1000Research*: Tang MK, Lo LM, Shi WT, Yao Y, Lee HSS & Lee KKH (2014), “Transient acid treatment cannot induce neonatal somatic cells to become pluripotent stem cells.” *F1000Research*.3:102 (DOI:10.12688/f1000research.4382). To date this on-line paper has been

viewed 14,848 times and downloaded 1,402 times. The on-line publication was cited in a 2015 *Nature* article in which seven of the largest international stem cell laboratories confirmed that STAP cells did not exist, some 17 months following Lee's publication. (Ref. 5.1)

During his attempt in replicating the STAP work, Lee serendipitously found the use of fibroblast spheroid culture and 3 small molecules to induce human fibroblasts to express pluripotent genes Oct4, Sox2 and Nanog. Consequently, a patent was filed and granted for this discovery in USA. (Ref. 5.9). In 2014, Lee acquired the license for the invention from his University and started a biotech company, Stapworks Stem Cell Limited, in order to commercialise the invention (<https://www.stapworks.com.hk/>). The company currently has two full time employees involved with the marketing, distribution and sales of its "Progammin" stem cells kit. To date, four kits have been sold to Yonsei University, South Korea and University of Hong Kong at US\$2,000 each. In addition, kits have been sent to University of Southampton (2X), Oslo (1X) and Oxford (1X) as collaborations to establish whether it could be used to rejuvenate senescence mesenchymal stem cells (the inability to generate sufficient quality of cultured mesenchymal stem cells because of cellular senescence is a major roadblock in the biotech industry.)

The STAP replication attempt was the inspiration in the making of the "SKY ON FIRE" movie, premiering 25-11-2016 in Hong Kong <http://www.imdb.com/title/tt6157600/>. The movie's credits include Lee as the "stem cell consultant". Actor Jim Chim plays Professor Lee's character in this action packed movie. The IMDb rating was 4.7 and the first 3 days taking was CNY 31 million.

The STAP saga has generated public awareness of issues relating to scientific integrity, bioethics and the powerful role of social media in science. Lee's replication attempt has been documented in several bioethics-associated publications. Durham University used the STAP saga and the *F1000Research* paper in teaching bioethics (Lancaster, C. 2016 The acid test for biological science. STAP cells, trust, and replication. *Science and Engineering Ethics* 22(1): 147-167). Lee's STAP replication effort and subsequent experience in getting the result published has also made the case for mainstream journals to publish "non-reproducible results" (<http://www.sciencemag.org/news/2016/02/if-you-fail-reproduce-another-scientist-s-results-journal-wants-know>). It has also stimulated a rethinking of other appropriate modes for scientific publishing, especially in the area of open peer review (e.g., *F1000Research*) versus traditional forms of publishing (e.g., *Nature*). Lee's *F1000Research* paper has been one of the catalysts in the growing acceptance of "negative" results by a number of journals (e.g., *Stem Cell Reports*).

(5) **Sources to corroborate the impact** (indicative maximum of 10 references)

High impact journal citing Prof. Lee's research

- 5.1 De Los Angeles A, Ferrari F, Fujiwara Y, Mathieu R, Lee S, Lee S, Tu HC, Ross S, Chou S, Nguyen M, Wu Z, Theunissen TW, Powell BE, Imsoonthornruksa S, Chen J, Borkent M, Krupalnik V, Lujan E, Wernig M, Hanna JH, Hochedlinger K, Pei D, Jaenisch R, Deng H, Orkin SH, Park PJ, Daley GQ. (2015) Failure to replicate the STAP cell phenomenon. *Nature* 525(7570):E6-9. DOI: 10.1038/nature15513

Prof. Lee's international press interviews and release

- 5.2 Hong Kong scientist casts doubt on stem-cell study
The Wall Street Journal March 13 2014
- 5.3 Stem cell 'breakthrough data inappropriately handled'
BBC News March 14 2014

- 5.4 Major stem cell study debunked on scientific social network
WIRED *March 14 2014*
- 5.5 Reliability of stem cell ‘breakthrough’ questioned
NHS choices *March 17 2014*
- 5.6 Acid test
The Economist *March 22 2014*
- 5.7 More STAP Trouble
The Scientist *March 25 2014*
- 5.8 Stem cell scientist Haruko Obokata found guilty of misconduct
The Guardian *April 1 2014*

Patent

- 5.9 **Lee KKH** Combinational Use of Mechanical Manipulation and Programin Derivatives to Increase OCT4, SOX2, or NANOG Expression in Fibroblasts. **US Patent, No. 10,167,451 B2.** issued by the USPTO on 1 January 2019.

Television Production casting Prof. Lee

- 5.10 STAP Stem Cells Controversy. **Fuji Television Network, Inc, Japan**, “Mr Sunday, (a popular public entertainment and communication program)” Broadcasted 4th April 2014 (Video recording included)
https://drive.google.com/file/d/1gYH3BMK_QK8G2jZUH6WcO1xT8CpNvfbv/view?usp=drive_web