A way to wipe a cell's memory

Cells that have been artificially reprogrammed into states similar to embryonic stem cells — known as induced pluripotent stem cells — can bear a memory of their previous history. An innovative method that incorporates a step mimicking early development yields pluripotent cells that more closely resemble those in embryos, both on a molecular and functional level.

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The problem

Cells of the body, called somatic cells, can be artificially reprogrammed into induced pluripotent stem (iPS) cells that can then differentiate into other cell types. This reprogramming requires all the biochemical (or 'epigenetic') modifications on a cell's DNA and associated proteins to be reconfigured until they resemble those of embryonic stem (ES) cells, which are similar to cells found in the early embryo. Collectively, these modifications are known as the epigenome.

However, compared with ES cells, iPS cells generated through large-scale conventional reprogramming show considerable epigenetic and functional differences[1–3]. These arise from both a residual epigenetic memory of the cell's previous somatic state and from newly introduced epigenetic aberrations, but their underlying mechanisms are not fully understood. The differences limit the accuracy of epigenetic reconfiguration during reprogramming and can be inherited by the cells formed from differentiated iPS cells, with unpredictable consequences. What's needed is a way to reduce these epigenetic differences so that human iPS cells are of maximal use in disease modelling, drug screening and therapies.

The discovery

Conventionally generated, 'primed' iPS cells resemble those in the embryo after implantation, but technical advances now enable the generation of 'naive' iPS cells that are more similar to cells in the pre-implantation embryo. The naive state also exhibits widespread epigenome erasure[4] — as occurs in mammalian cells during early embryonic development, before epigenetic modifications are re-established. By examining human somatic cells at various stages of reprogramming, we pinpointed when epigenetic aberrations emerge as they become primed iPS cells, and showed that a five-day exposure to the cell-culture conditions used to generate naive iPS cells can extensively erase the epigenome without resulting in such abnormalities. We used this knowledge to devise a method we call transient-naive-treatment (TNT) reprogramming that emulates the epigenetic 'reset' seen in early embryonic development.

Our TNT-reprogramming strategy corrected epigenetic memory and aberrations, resulting in human iPS cells that more closely resemble human ES cells on a molecular and functional level than do conventionally generated human iPS cells (Fig. 1). We demonstrated that the epigenetic memory in conventional human iPS cells is concentrated in domains of the DNA–protein complex called chromatin that repress gene expression in the cell type of origin. TNT reprogramming reconfigures these domains to a state similar to that in human ES cells, and corrects the inappropriate expression of genes and DNA sequences called transposable elements that is seen in conventional human iPS cells (Fig. 1). Crucially, TNT reprogramming improves differentiation of human iPS cells into various specialized cell types, even when iPS cells are generated from different types of somatic cell.

The implications

The TNT-reprogramming approach could substantially advance cell-based therapies and disease models, because it generates human iPS cells that, compared with conventional human iPS cells, are more similar to human ES cells and can give rise to cells that accurately resemble target ones. The strategy also addresses the problem of transposable-element overexpression seen in conventionally generated human iPS cells. This is important because, if unresolved, such overexpression could have severely detrimental consequences for cell therapies in the long run[5]. Hence, we predict that TNT reprogramming will establish a new benchmark for biomedical and therapeutic uses and offer an innovative system for investigating epigenetic memory.

Although particular epigenetic modifications are associated with the residual epigenetic memory and aberrations seen in conventional iPS cells, the mechanisms that cause and correct them remain unknown. For example, residual epigenetic modifications are often concentrated in repressed regions of the genome that are associated with the lining of the nucleus, but we still do not understand why they resist reprogramming. Similarly, although we know when the epigenetic aberrations are generated during conventional reprogramming, we do not know how they are established.

We wish to resolve these and other mechanistic points, including: why some genomic regions are more resistant to reprogramming; which elements of the naive cell-culture conditions underlie the effects of TNT; and how epigenetic modifications are erased by TNT. Finally, we want to study the extent to which epigenetic memory remains in other cell-fate paradigms, such as during development.

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EXPERT OPINION

Buckberry *et al.* describe a modified protocol for cell reprogramming to obtain human induced pluripotent stem (iPS) cells that are epigenetically and functionally equivalent to human embryonic stem cells. Epigenetic aberrations and differentiation defects in human iPS cells have previously been reported in the literature, and despite both representing hindrances for clinical applications of human iPS cells, have remained unsolved. Therefore, the protocol could potentially have a high impact on the field." (CC BY 4.0)

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FIGURE

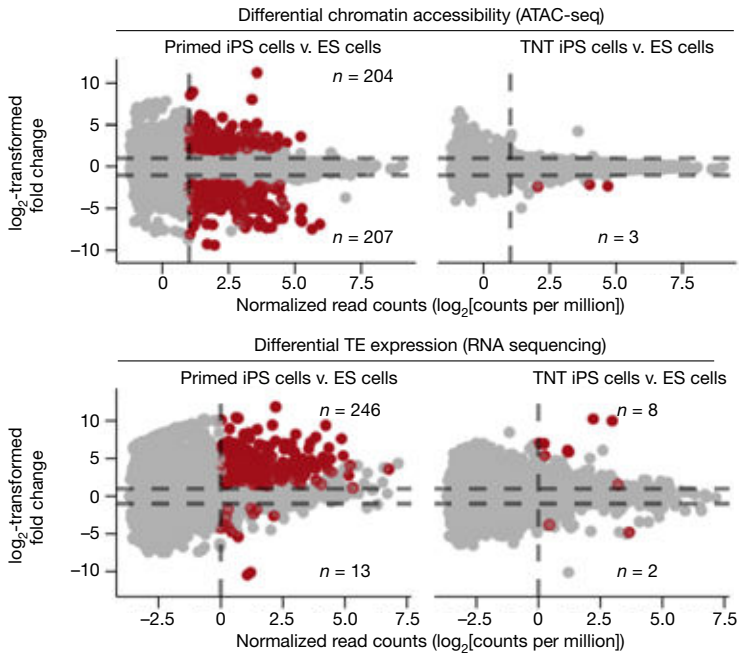

Figure 1 | Reducing differences between reprogrammed pluripotent cells and embryonic stem cells. Cells of the body can be reprogrammed into induced pluripotent stem (iPS) cells, which differentiate into various cell types. However, conventionally generated, 'primed' human iPS cells retain and develop modifications to the chromatin DNA–protein complex that differentiate them from human embryonic stem (ES) cells. A method called TNT reprogramming reduces these differences. A technique called ATAC-seq reveals that, at hundreds of genomic regions in primed human iPS cells, the chromatin is too relaxed or too compacted compared with human ES cells (red circles in upper left graph; grey circles show regions that are not statistically different from those in human ES cells, or where normalized read counts were too low to compare). Almost all of these differences were corrected in TNT human iPS cells (upper right graph). RNA sequencing revealed that primed human iPS cells show higher expression of hundreds more short DNA segments called transposable elements (TEs) than human ES cells do (red circles in lower left graph); this activation of expression is almost completely corrected in TNT human iPS cells (lower right graph). Buckberry, S. *et al./Nature* (CC BY 4.0).

BEHIND THE PAPER

While we were postdoctoral researchers in the United States, we independently reported the existence of epigenetic memory and aberrations in mouse and human induced pluripotent stem cells[1,2]. Coincidentally, we had both also decided to start our own labs in Australia, relocating to Melbourne and Perth in 2011. On learning this, we exchanged e-mails and, when we met, decided to try to solve this problem together, rather than to compete. Since then, we have been working together on various aspects of the cell-reprogramming field. It was at the Lorne Genome Conference in 2015 that we devised the experimental approaches that we would use to better explore the problem in this study, and we have been working on it since then. Research can take a long time, but it is particularly satisfying to have worked together on this and come full circle to address the problem we both identified many years ago!

J.M.P. and **R.L.**

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FROM THE EDITOR

The authors' comprehensive documentation of epigenetic marks in cells that have been reprogrammed to naive and primed pluripotent states is impressive. This enabled them to develop a simple and easily implementable protocol that improves differentiation of human induced pluripotent stem cells, and will greatly facilitate both reproducibility in basic research and translation to the clinic.

Editorial team, *Nature*