iPSC don´t Forget their Origins.

By Stuart P. Atkinson

Our latest news section recently highlighted the publication of two advance articles from Nature and Nature Biotechnology which suggest that induced pluripotent stem cells (iPSCs) retain memories of the differentiated cell type from which they were derived; their cell of origin. Understandably, this raises several questions about the comparability of iPSCs to human embryonic stem cells (hESCs), particularly since the concept of epigenetic memory has arisen in the production of cloned mammals using somatic cell nuclear transfer. Given the importance of this topic, here we provide a more in-depth discussion of these two articles.

The first article from the laboratories of George Daley and Andrew Feinberg started out as a study directed towards analyzing haematopoietic stem cells (HSCs) generated from mouse nuclear transfer and embryo-derived ESCs (ntESCs and fESCs respectively) and low passage iPSCs derived from fibroblasts (F-iPSCs) or blood (B-iPSCs). Initial studies however found an intriguing difference in the haematopoietic differentiation propensities of these cells, turning the authors attention towards this phenomenon. The type of cells chosen for the initial intended research project allowed some exciting comparative findings, when applied in this new direction. Analysis showed that while both iPSC lines gave robust multi-lineage teratomas, other accepted measures of pluripotency were lacking when compared with ntESCs and fESCs. Furthermore, they also discovered that while both ntESCs and fESCs could differentiate readily down the haematopoietic and osteogenic lineages, F-iPSCs and B-iPSCs preferentially differentiated towards a specific lineage linked to their cell of origin; B-iPSCs down the blood lineage and F-iPSCs down the osteogenic pathway.

Analysis of differentially methylated regions (DMRs) of DNA throughout the genome suggested a mechanism by which this might happen. While ntESCs and fESCs where very similar (229 DMRs), the F-iPSCs and B-iPSCs differed to fESCs (3,349 and 516 DMRs), and the F- iPSCs and B-iPSCs were also very dissimilar to each other (5,202 DMRs). Interestingly, of the top 24 DMRs which distinguished F- iPSCs from B-iPSCs, 11 of these were linked to haematopoiesis and 3 to ostegenesis, with 10 of the 11 haematopoiesis-associated DMRs hypermethylated and therefore silent in the F-iPSCs. DMRs associated with haematopoietic transcription factors were also preferentially methylated in F-iPSC whilst those associated with fibroblast-specific genes were preferentially methylated in B-iPSCs. Similar analysis also

showed lineage bias when comparing iPSC from B lymphocytes (Bl-iPSCs) and neural progenitors (NP-iPSCs). These iPSCs were secondary in nature, in that they were derived from mice chimaerised with iPSCs carrying dox-inducible transgenes and a Nanog-eGFP reporter gene to assay for the pluripotent nature of these cells. Analysis of DMRs in secondary iPSCs showed some similarity between NP-iPSCs and fESCs, but that Bl-iPSCs were very different, suggesting that the NPCs were more fully reprogrammed than the B-lymphocytes, perhaps due to their differences in stage of differentiation. However, analysis of hypermethylated DMRs showed differences between NP- iPSCs and Bl-iPSCs relative to fESCs, further highlighting the epigenetic dissimilarity between 'true' ESCs and these iPSCs, even though they were shown to be pluripotent in nature. All of these exciting data strongly suggests that DNA methylation signatures harbored from the cell of origin can influence (and thereby limit) the differentiation potential of iPSCs.

Further experiments attempted to re-write or chemically modify the epigenetic environment in order to discover whether DNA methylation patterns arising from the cell of origin could be erased. Firstly, the authors differentiated NP-iPSCs towards the haematopoietic lineage then reprogrammed the resultant differentiated cells to pluripotency (B-NP-iPSC). When compared to a NP-iPSC line that had been differentiated towards a neural stem cell lineage (NSC-NP-iPSC), B-NP-iPSC demonstrated higher haematopoietic differentiation potential. This interesting experiment again strongly suggests that the memory of cell of origin can influence lineage potential, even if that origin is modified through differentiation (from a neural origin to a blood origin). Furthermore, treatment of NP-iPSCs with chromatin-modifying drugs allowed enhanced haematopoietic differentiation, fortifying the notion that changes to the epigenetic landscape of the genome can influence iPSC lineage differentiation. Overall, these data suggest transcription-factor based reprogramming of somatic cells is not entirely complete, which will likely have important implications for future therapeutic use of the iPSCs.

The second article, from the lab of Konrad Hochedlinger, reports similar findings with an added twist! They studied secondary iPSCs derived from tail tip-derived fibroblasts (TTFs), splenic B-cells (B), bone marrow-derived granulocytes (Gra) and skeletal muscle precursors (SMPs), which utilizing various accepted means were deemed to be pluripotent. However, initial transcriptional analysis found that Gra-iPSCs and SMP-iPSCs could be distinguished

simply by using markers of the cell of origin for the two lines, suggesting retainment of transcriptional memory in these iPSCs. Notably, subsequent analysis showed major transcriptional differences between all four iPSC types, and notably, that the 100 genes showing the greatest differences between SMP- and Gra-iPSCs were implicitly linked to the cell of origin. Some differences, similar to the transcriptional analysis, were observed following analysis of DNA methylation at gene promoters, but the differences became more obvious when epigenetic analysis was extended to histone modifications. The authors found that previous transcriptional perturbations in the iPSC studied were linked to permissive or repressive histone modifications dependent on their transcriptional status. Similar to the previous study, distinct differences in differentiation potential were observed between the iPSCs, with TTF-iPSC and SMP-iPSC differentiating poorly down the haematopoietic lineage compared with B-iPSCs and Gra-iPSCs, again demonstrating that the influence of the cell of origin for lineage specification in iPSCs.

Overall, it seems that in low-passage iPSCs the transcriptional, epigenetic and differentiation differences are linked to the cell of origin, but, excitingly, this study goes on to demonstrate that continuous passaging of iPSCs leads to the erasure of these differences. They demonstrate that early-passage (passage 4) iPSCs are distinguishable transcriptionally, epigenetically and by differentiation-potential, but by passage 16, these differences are abrogated. Subsequent analysis suggests that common changes occur during the passage of iPSCs irrespective of cell of origin and further the authors also rule out the out-growth of a rare population from within the iPSCs, suggesting that this 'dilution' of cell-of-origin memory happens in all iPSCs.

This suggests that the "memories" which seem to haunt the authors in the first paper are thoroughly exorcised by the second, although questions still abound. It is noted by the authors of the second paper that the previously observed silencing of the imprinted Dlk-Dio3 cluster is not altered by passaging of cells, suggesting that not *all* epigenetic modifications are reset. So, is this a special case or are imprinted regions likely to be protected somehow from being fully reprogrammed? Further, miRNA and other small RNA species are not studied in these papers. The authors do however suggest a potential mechanism by which iPSCs become more alike after many passages, this being a "slow process, potentially facilitated by a positive feedback mechanism that gradually resolves the residual cell-of-origin-specific epigenetics marks and transcriptional patterns." So is it the case that young iPSCs are simply not up to the job early in their life, but learn to become

fully reprogrammed iPSCs with time? This then suggests that only late stage iPSCs should be considered useful for clinical and research purposes when in a fully reprogrammed state. Also, one observation from hESC biology is that different cell lines have different propensities for differentiation down specific lineages. This may be due to genetic rather than epigenetic differences since these lineage potential differences do not appear to change much with increasing passage number. Thus such intrinsic genetic differences may eventually be observed with iPSCs even after their epigenetic memory has been erased by prolonged culture.

The erasure of the epigenetic memory from the cell of origin is an exciting finding that was not explored in the first study, turning an initially bad piece of news for regenerative medicine into a novel finding relating to the mechanisms and underlying complexity behind somatic cell reprogramming. One must assume that similar studies, understandably not as complex and as full as for the mouse system, will be forging ahead for human iPSCs as we read this now.

References

Polo, J.M., S. Liu, M.E. Figueroa, W. *et al.*
Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nat Biotechnol.

Kim, K., A. Doi, B. Wen, K. *et al.*
Epigenetic memory in induced pluripotent stem cells.
Nature.

Stadtfeld, M., Apostolou, E., Akutso, H., *et al*.
Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells.
Nature 465, 175–181 (2010).

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