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Epigenetic Aberrations Are Not Specific to Transcription Factor-Mediated Reprogramming

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SUMMARY

Somatic cells can be reprogrammed to pluripotency using different methods. In comparison with pluripotent cells obtained through somatic nuclear transfer, induced pluripotent stem cells (iPSCs) exhibit a higher number of epigenetic errors. Furthermore, most of these abnormalities have been described to be intrinsic to the iPSC technology. Here, we investigate whether the aberrant epigenetic patterns detected in iPSCs are specific to transcription factor-mediated reprogramming. We used germline stem cells (GSCs), which are the only adult cell type that can be converted into pluripotent cells (gPSCs) under defined culture conditions, and compared GSC-derived iPSCs and gPSCs at the transcriptional and epigenetic level. Our results show that both reprogramming methods generate indistinguishable states of pluripotency. GSC-derived iPSCs and gPSCs retained similar levels of donor cell-type memory and exhibited comparable numbers of reprogramming errors. Therefore, our study demonstrates that the epigenetic abnormalities detected in iPSCs are not specific to transcription factor-mediated reprogramming.

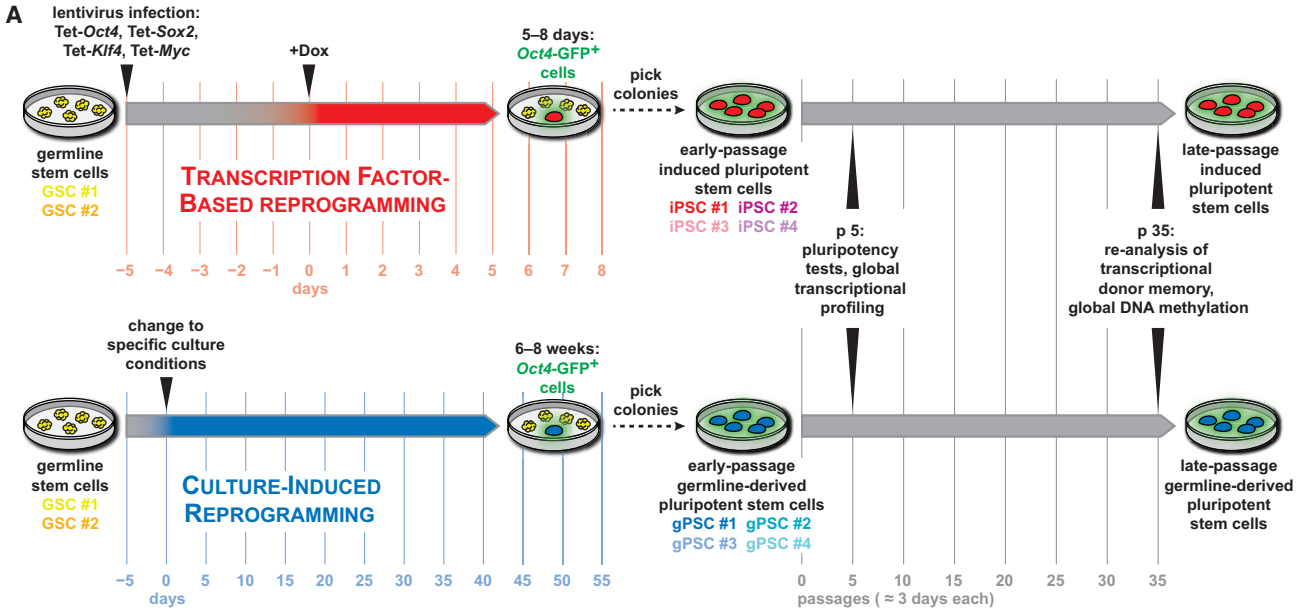
INTRODUCTION

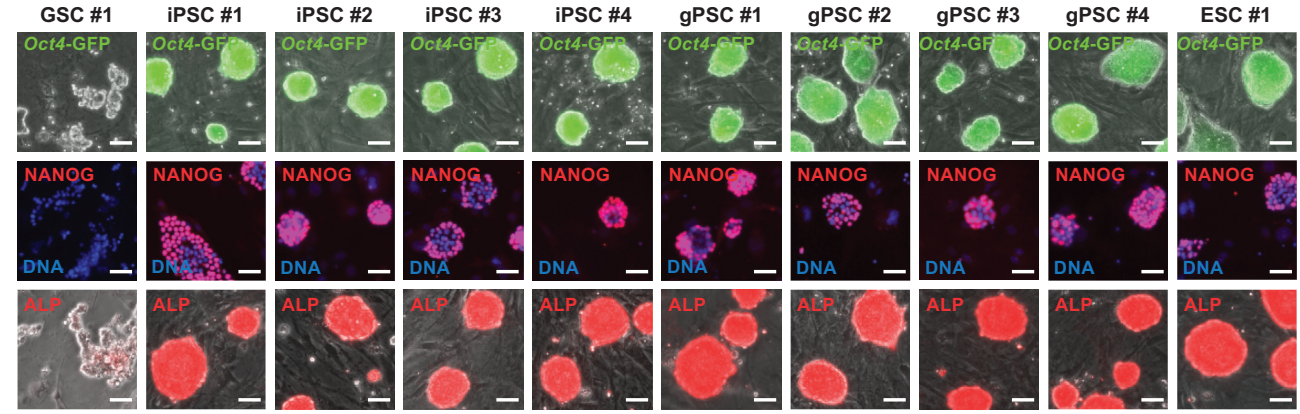
Previous studies have reported that induced pluripotent stem cells (iPSCs) retain epigenetic traits of the tissue of origin and accumulate DNA methylation errors during the reprogramming process (Kim et al., 2010; Ma et al., 2014). However, whether these epigenetic abnormalities are a consequence of cellular reprogramming per se or are specific to the iPSC technology remains controversial. A previous study has shown that most of the abnormalities found in iPSCs are introduced during the reprogramming process (Ma et al., 2014). Indeed, iPSCs exhibit more aberrations than pluripotent cells obtained through somatic cell nuclear transfer (SCNT) (Ma et al., 2014). In this study, we used a third reprogramming method to investigate whether iPSC epigenetic errors are a consequence of the ectopic expression of transcription factors. Germline stem cells (GSCs) are the only adult cell type that can be converted into pluripotent stem cells, termed germline pluripotent stem cells (gPSCs), under specific culture conditions (Ko et al., 2009). Thus, we compared GSC-derived iPSCs with gPSCs at the transcriptional and epigenetic level. Global gene expression and genome-wide DNA methylation analysis confirmed that GSC-derived iPSCs and gPSCs exhibit similar levels of donor memory and de novo reprogramming errors. Therefore, our results indicate that epigenetic aberrations are not specific to transcription factor-mediated reprogramming.

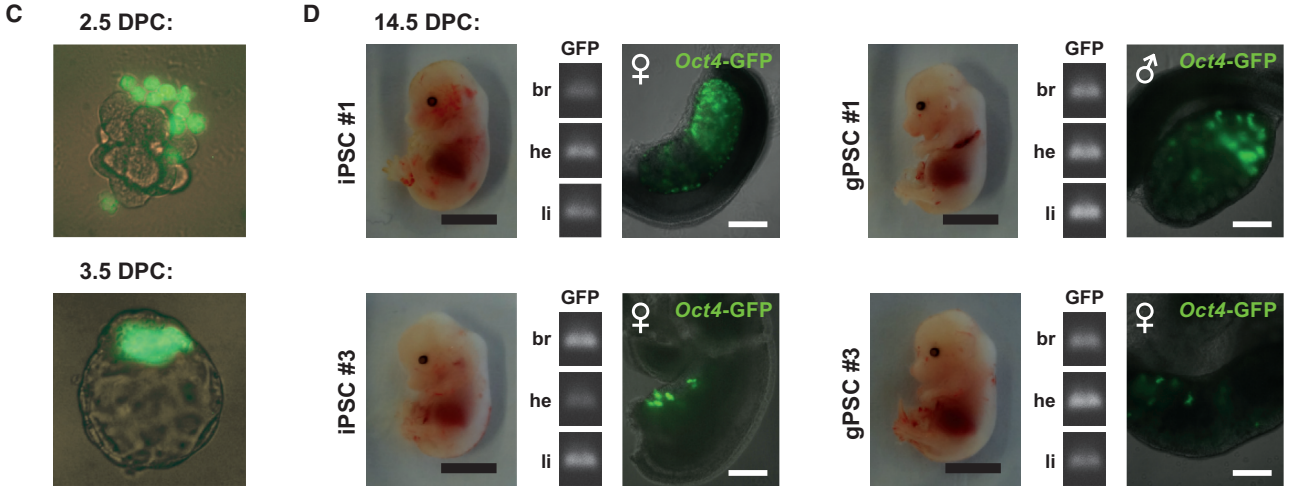
RESULTS

Conversion of GSCs to Pluripotency Using Two Different Reprogramming Methods

We derived two GSC lines, named GSC #1 and #2, from mice containing an *Oct4*-GFP reporter transgene (Yoshimizu et al., 1999). Next, we reprogrammed both GSC lines to pluripotency using two different methods (Figure 1A). First, doxycycline-inducible lentiviruses coding for *Oct4*, *Sox2*, *Kfl4*, and *Myc*, together with a reverse tetracycline transactivator (Brambrink et al., 2008), were used to generate iPSCs from both GSC lines. *Oct4*-GFP-positive colonies emerged 5–8 days after transgene induction. Although a previous study reported the inability to generate GSC-derived iPSCs using constitutively expressed lentiviruses (Morimoto et al., 2012), we were able to reprogram GSCs into iPSCs using inducible lentiviruses (for details see Supplemental Experimental Procedures). In parallel, iPSCs were generated from fibroblasts (Fib) from the same mouse line and following the same procedure. Interestingly, Fib-iPSC colonies were not observed until 10–20 days after doxycycline induction, demonstrating that GSCs are reprogrammed significantly faster than other somatic cell types. As previously reported, the stoichiometry of the reprogramming factors influences the iPSC generation rate. Indeed, a high relative level of both *Oct4* and *Klf4* combined with a low relative level of *Sox2* and *Myc* has been described to increase the reprogramming efficiency (Tiemann et al., 2011). Accordingly, GSCs were







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