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-OH No! hiPSCs Misplace Their 5hmCs

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hiPSCs and hESCs are thought to display subtle genetic and epigenetic variability. Recently in *Nature Cell Biology*, Wang et al. (2013) demonstrated a role for TET1 during reprogramming of human cells and showed that hiPSCs lack appropriate 5hmC marks in subtelomeric regions, contributing to epigenetic variation common to hiPSCs.

The ability to reprogram cell fate by the overexpression of a handful of genes has opened the door to limitless possibilities for modeling development or diseases in vitro and eventual patient-specific clinical applications. Yamanaka and colleagues first described this paradigm by overexpressing four transcription factors in fibroblasts, leading to their conversion to a pluripotent state. Since then, pluripotent cells derived by induction (induced pluripotent stem cells, or iPSCS) have been extensively compared to their embryo-derived counterparts (embryonic stem cells, or ESCs). Many studies have identified epigenetic and transcriptional differences between these types of pluripotent stem cells (Chin et al., 2009; Lister et al., 2011; Ohi et al., 2011; Ruiz et al., 2012), while others have suggested that these differences are no more dramatic than those found when comparing different ESC lines (or iPSC lines) to each other (Bock et al., 2011). Ultimately, high-quality human iPSCs and ESCs could be functionally equivalent, while potentially still distinguishable at the molecular level depending on the resolution of the analysis and the number of lines analyzed.

In the present study, Wang et al. (2013) exploit high-resolution analyses of DNA methylation to confirm and extend previous findings that hiPSCs are distinguished from hESCs by altered methylation status of subtelomeric DNA. Previously, three groups provided compelling evidence that hiPSCs appear to have aberrant patterns of 5-methyl-Cytosine (5mC) modification within subtelomeric DNA regions (Lister et al., 2011; Ohi et al., 2011; Ruiz et al., 2012). This patterning was thought to result from inefficient erasing and/or rewriting of the methylome during reprogramming, reflecting an epigenetic memory of the somatic state from which they were derived. However, since these previous studies relied upon traditional bisulfite sequencing, they did not distinguish 5mC from 5-hydroxymethyl-Cytosine (5hmC), a mark that has recently been shown to be a signature aspect of the methyl-DNA repertoire and important for gene regulation. Wang et al. first found that TET1, a key enzyme that converts 5mC to 5hmC, is strongly induced during reprogramming, and that blocking its expression abrogated the reprogramming process. This suggests that the selective conversion of 5mC to 5hmC is important for acquisition of the pluripotent state (Wang et al., 2013). Similar results were obtained previously for Tet1 and Tet2 in murine reprogramming (Koh et al., 2011). Interestingly, knocking down TET1 in human pluripotent cells had little effect on the pluripotent state, suggesting that the key role for TET1 occurs during the initial conversion of 5hmC during reprogramming and does not involve the maintenance of this mark (Wang et al., 2013).

Extensive profiling of 5hmC in fibroblasts, hiPSCs, and hESCs demonstrated that, out of 372,423 regions that were enriched for 5hmC marks throughout the genome, just 113 (0.03%) could be classified as differentially hydroxyl-methylated regions (DhMRs) when drawn from comparisons between hiPSCs and hESCs. The finding that such a tiny fraction of the genome appeared to be differentially methylated is consistent with previous reports and is further evidence of the remarkably faithful process that is induced upon introduction of the Yamanaka factors. On the other hand, 93% of these DhMRs were hypohydroxymethylated, which would indicate that the reprogramming process appears to specifically fail to convert 5mC to 5hmC in certain portions of the genome, leaving these regions in a state more typical of the somatic cells from which they came. Furthermore, the majority of these DhMRs tended to be localized to subtelomeric regions of the genome.

These findings are interesting in light of previous studies showing similar patterns of 5mC in subtelomeric regions of hiPSCs (Lister et al., 2011; Ruiz et al., 2012). Remarkably, a list of nine genes observed to be differently expressed in hiPSCs versus hESCs by Ruiz et al. (2012) highly overlaps with the list of summarized hypomethylation hotspots presented in Wang et al. The fact that multiple labs using distinct cell lines came to similar conclusions is strong evidence that these subtelomeric regions are indeed hotspots of reprogramming and warrant further consideration as possible proxies for defining the quality of PSCs at the molecular level. This metric could prove to be useful because no assay currently exists to quantitatively assess the quality of human pluripotent stem cells. Regardless, the methylation status of these hotspots across human pluripotent stem cell lines is clear evidence that, at a minimum, hiPSCs exhibit significantly more epigenetic variation than existing hESCs.

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The data in Wang et al. might appear to be confounding to previous studies that did not find consistent DhMRs between hESCs and hiPSCs. The simplest explanation is that the molecular differences between these types of pluripotent stem cells are quite subtle (just 0.03% of 5hmC-enriched regions in Wang et al., 2013). Therefore, studies in which many lines have been compared at low resolution (with Reduced Resolution Bisulphite Sequencing or DNA Methylation Arrays) did not identify consistent differences (Bock et al., 2011; Nazor et al., 2012), while those that used high resolution (single nucleotide) analyses on fewer lines have reported differences (Lister et al., 2011; Ohi et al., 2011; Ruiz et al., 2012; Wang et al., 2013). Regardless, the more compelling issue is why these subtelomeric domains are apparently difficult to appropriately methylate during reprogramming. Furthermore, can one take advantage of this observation to learn something about the basic mechanisms of how the Yamanaka factors drive this transformation? Numerous epigenetic barriers to reprogramming have recently been identified, partially explaining the very low inefficiency of the process (Watanabe et al., 2013). Wang et al. suggest that activity of TET1 represents yet another barrier to proper reprogramming to the pluripotent state.

The replication and organization of telomeres presents a serious engineering problem for cells. The ends of chromosomes require their own unique machinery to preserve the length and integrity of telomeres, while isolating the subtelomere domain from such machinations. Among the panoply of reorganization events that must occur during reprogramming is the reestablishment of telomere length by telomerase. Another recent paper demonstrated that TRF1, a component of the shelterin complex that maintains telomere integrity, was required for reprogramming (Schneider et al., 2013). Together with those of Wang et al., these findings suggest that reorganizing telomeres during reprogramming is not just a matter of restoring their length, but also requires the activity of the shelterin complex and epigenetic remodeling of the subtelomeric domain. This latter reorganization appears to fall somewhat short in hiPSCs, affecting the expression of several genes in these regions (TCERG1I, TMEM132D, etc.) (Chin et al., 2009; Ruiz et al., 2012; Wang et al., 2013). The key unresolved issue is whether the small degree of hypomethylation observed in hiPSCs has a functional significance or is inconsequential. Regardless, one should take into account the increased degree of epigenetic variability across hiPSC lines when modeling disease or development in vitro.

REFERENCES

Bock, C., Kiskinis, E., Verstappen, G., Gu, H., Boulting, G.L., Smith, Z.D., Ziller, M., Croft, G.F., Amoroso, M.W., Oakley, D.H., et al. (2011). Cell *144*, 439–452.

Chin, M.H., Mason, M.J., Xie, W., Volinia, S., Singer, M., Peterson, C., Ambartsumyan, G., Aimiuwu, O., Richter, L., Zhang, J., et al. (2009). Cell Stem Cell *5*, 111–123.

Koh, K.P., Yabuuchi, A., Rao, S., Huang, Y., Cunniff, K., Nardone, J., Laiho, A., Tahiliani, M., Sommer, C.A., Mostoslavsky, G., et al. (2011). Cell Stem Cell *8*, 200–213.

Lister, R., Pelizzola, M., Kida, Y.S., Hawkins, R.D., Nery, J.R., Hon, G., Antosiewicz-Bourget, J., O'Malley, R., Castanon, R., Klugman, S., et al. (2011). Nature *471*, 68–73.

Nazor, K.L., Altun, G., Lynch, C., Tran, H., Harness, J.V., Slavin, I., Garitaonandia, I., Müller, F.J., Wang, Y.C., Boscolo, F.S., et al. (2012). Cell Stem Cell *10*, 620–634.

Ohi, Y., Qin, H., Hong, C., Blouin, L., Polo, J.M., Guo, T., Qi, Z., Downey, S.L., Manos, P.D., Rossi, D.J., et al. (2011). Nat. Cell Biol. *13*, 541–549.

Ruiz, S., Diep, D., Gore, A., Panopoulos, A.D., Montserrat, N., Plongthongkum, N., Kumar, S., Fung, H.L., Giorgetti, A., Bilic, J., et al. (2012). Proc. Natl. Acad. Sci. USA *109*, 16196–16201.

Schneider, R.P., Garrobo, I., Foronda, M., Palacios, J.A., Marión, R.M., Flores, I., Ortega, S., and Blasco, M.A. (2013). Nat Commun *4*, 1946.

Wang, T., Wu, H., Li, Y., Szulwach, K.E., Lin, L., Li, X., Chen, I.P., Goldlust, I.S., Chamberlain, S.J., Dodd, A., et al. (2013). Nat. cell Biol. *15*, 700–711.

Watanabe, A., Yamada, Y., and Yamanaka, S. (2013). Philos. Trans. R. Soc. Lond. B Biol. Sci. *368*, 20120292.