CellPress

Of Mice and Man: Differential DNMT Dependence in Mammalian ESCs

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http://dx.doi.org/10.1016/j.stem.2015.04.009

Liao et al. (2015) recently reported on the effects of disrupting DNA methyltransferase activity in human embryonic stem cells (hESCs). This work highlights key differences between mammalian ESC models upon the loss of these essential proteins and provides comprehensive base resolution methylome maps of DNMT targets during human development.

Cytosine DNA methylation is a heritable chemical modification of the DNA molecule, predominantly associated with transcriptional repression and essential for mammalian development. In mammals, the DNA methylation mark is deposited and maintained through cell division by the family of DNA methyltransferase (DNMT) enzymes. The major mammalian DNMT family members are DNMT1, required for maintenance of DNA methylation through genome replication, and DNMT3A/DNMT3B, which generally perform de novo DNA methylation (Jurkowska et al., 2011). Interestingly, overlaps in functionality have been reported between DNMT1 and DNMT3 enzymes. A new study by Liao et al. (2015) addresses how the precise targeted ablation of these proteins' functions affects the differentiation capacity of human embryonic stem cells (hESCs) and highlights the different effects of DNMT disruption between mouse and human ESCs.

In their study, Liao et al. employed the CRISPR/Cas9 genome editing system to systematically disrupt the catalytic domain of DNMT1, DNMT3a, and DNMT3b proteins in an hESC line, HUES64. They demonstrate that the *DNMT3A*$^{-/-}$; *DNMT3B*$^{-/-}$ double knockout cells are fully viable and able to differentiate into all the three germ layers (ectoderm, endoderm, and mesoderm) as assessed by both in vitro and in vivo assays (Figure 1). This is consistent with previous studies undertaken in mouse ESCs (mESCs), where single *DNMT3A* or *DNMT3B* knockouts were able to successfully differentiate into erythroid and myeloid lineages and form teratomas (Chen et al., 2003; Jackson et al., 2004). However, mouse *DNMT3A*$^{-/-}$; *DNMT3B*$^{-/-}$ double knockout ESCs only displayed full differentiation capacity at earlier passages due to the progressive loss of methylation that affected later cultures (Jackson et al., 2004). This lack of differentiation potential does not appear to hold true for *DNMT3A*$^{-/-}$or *DNMT3B*$^{-/-}$ hESCs, which were able to form teratomas and embryoid bodies even at later passages, despite reduced DNA methylation levels. However, perhaps the most striking difference between mESCs and hESCs is the hESC-specific sensitivity toward the loss of DNMT1 activity (Figure 1). While Liao et al. were not able to obtain *DNMT1*$^{-/-}$ hESC clones despite the high efficiency of *DNMT1*$^{+/-}$ heterozygous line generation, this requirement appears to be absent from mESCs, which surprisingly can be propagated in the absence of all three examined DNMTs (Tsumura et al., 2006). To further corroborate these interesting findings, Liao et al. applied an alternative, TALEN-based genome editing strategy that displayed the same inability to generate a *DNMT1*$^{-/-}$ hESC line. Consequently, to create a *DNMT1*$^{-/-}$ hESC line that would not result in immediate lethality, the authors designed an elegant rescue approach by introducing a doxycycline-responsive (Tet-Off) *DNMT1* cassette in hESCs before targeting the endogenous sequence by the CRISPR/Cas9 genome editing system. This resulted in the creation of a stable line that proliferated normally so long as the exogenous *DNMT1* cassette was being expressed. Addition of doxycycline caused rapid downregulation of the exogenous *DNMT1* and resulted in subsequent cell death, thereby confirming the DNMT1 requirement for hESC viability.

To interrogate the effects of DNMT function loss on the hESC DNA methylome, Liao et al. combined their powerful knockout model system with base resolution methylation profiling techniques. Whole-genome bisulfite sequencing of *DNMT3A*$^{-/-}$, *DNMT3B*$^{-/-}$, and *DNMT3A*$^{-/-}$; *DNMT3B*$^{-/-}$ cells allowed the identification of genomic targets specific to each DNMT3 protein. Whereas the majority of low CpG-density genomic regions appeared to be redundantly regulated by both DNMT3A and DNMT3B, regions of high CpG density such as CpG islands displayed preference for either DNMT3A or DNMT3B. Those CpG islands were enriched for Polycomb repressive complex 2 (PRC2) component binding sites such as SUZ12 and EZH2, a histone-lysine N-methyltransferase responsible for the deposition of H3K27me3. This suggests that a subset of regions targeted by DNMT3A or DNMT3B and hypermethylated in hESCs likely lose methylation during differentiation and become targets for Polycomb regulation. This is consistent with previous studies describing the cross-talk between DNA methylation and H3K27me3 and the mutual exclusivity of these marks on CpG islands in mESCs (Brinkman et al., 2012). Liao et al. also explored the perturbations of CH methylation, a previously described hallmark of hESCs (Ramsahoye et al., 2000), and confirmed that DNMT3A and DNMT3B are required for the propagation of this atypical methylation signature, with *DNMT3A*$^{-/-}$; *DNMT3B*$^{-/-}$ cells displaying a rapid loss of CH methylation that was not observed in the doxycycline-induced

DNMT1$^{-/-}$ system. Finally, to investigate the effects of DNMT loss on the generation of a defined cell lineage, Liao et al. took full advantage of their in vitro differentiation system and derived definitive endoderm lineages from wild-type and *DNMT3A*$^{-/-}$cells, observing a loss of DNA methylation in promoters and gene bodies of these cells, thereby exposing unique activities of DNMT3A during endoderm differentiation.

In their current study Liao et al. provide a powerful system for modeling the loss of DNMT enzymes during early stages of human development and describe unique epigenomic footprints specific to each DNMT enzyme. Such studies in hESC systems are highly informative given that targeted disruption of these processes cannot be conducted during human embryogenesis. However, to date it is unclear how different culture conditions of hESCs affect the molecular phenotypes observed upon the loss of DNA methylation machinery components. Recent studies in mESCs cultured in naive conditions suggest a highly distinct DNA methylome configuration of such cells (Habibi et al., 2013). It will be interesting to see whether the observed DNMT phenotypes can be recapitulated in naive hESCs, as the cell lines currently used by Liao et al. correspond to a somewhat more mature, epiblast state. Liao et al. also propose an enticing alternative hypothesis that can potentially explain some differences in DNMT requirements between humans and mouse. The authors calculated the DNMT1 fidelity of hESCs to be higher than that of mESCs, which could suggest that the contribution of DNMT3A and DNMT3B to maintenance DNMT activity is reduced when compared to mESCs. Nevertheless, intriguing parallels can be drawn between the current ESC work and insights obtained from vertebrate embryos. The global loss of DNA methylation in the early mammalian embryo (Smith et al., 2012), together with the reduced repression potential of DNA methylation during blastula stages of *Xenopus* embryogenesis (Bogdanovic et al., 2011), suggests reduced requirements for DNA methylation during vertebrate pluripotency. These insights are effectively mirrored in hESCs by the current study of Liao et al. where the hESCs lacking both DNMT3 enzymes can still differentiate into all three germ layers, in addition to previous work undertaken in mESCs (Chen et al., 2003; Jackson et al., 2004; Liao et al., 2015). The pioneering work by Liao et al. provides an important foundation for future studies that will aim to explore how the loss of DNMTs affects differentiation of hESCs into different human lineages and examine the molecular targets of different DNMT enzymes during such processes.

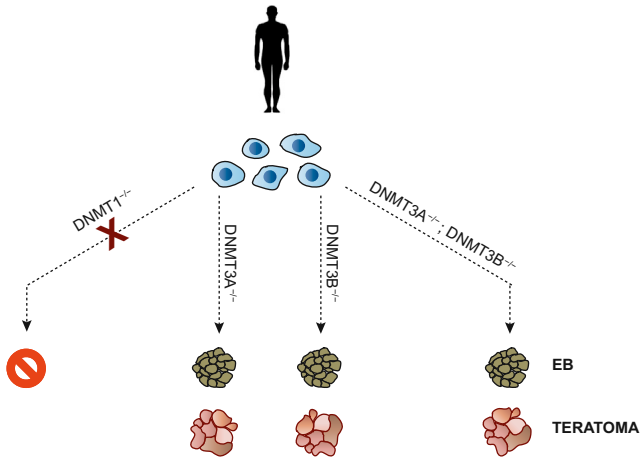

Figure 1. Differentiation Potential of hESC DNMT Mutant Lines
DNMT1 is essential for hESC viability, and disruption of DNMT1 in hESCs causes immediate cell death. Both DNMT3A and DNMT3B are dispensable for hESC maintenance and differentiation as exemplified by embryoid body (EB) and teratoma formation in *DNMT3A*$^{-/-}$, *DNMT3B*$^{-/-}$, and *DNMT3A*$^{-/-}$; *DNMT3B*$^{-/-}$ mutant lines.

REFERENCES

Bogdanovic, O., Long, S.W., van Heeringen, S.J., Brinkman, A.B., Gómez-Skarmeta, J.L., Stunnenberg, H.G., Jones, P.L., and Veenstra, G.J. (2011). Genome Res. *21*, 1313–1327.

Brinkman, A.B., Gu, H., Bartels, S.J., Zhang, Y., Matarese, F., Simmer, F., Marks, H., Bock, C., Gnirke, A., Meissner, A., and Stunnenberg, H.G. (2012). Genome Res. *22*, 1128–1138.

Chen, T., Ueda, Y., Dodge, J.E., Wang, Z., and Li, E. (2003). Mol. Cell. Biol. *23*, 5594–5605.

Habibi, E., Brinkman, A.B., Arand, J., Kroeze, L.I., Kerstens, H.H., Matarese, F., Lepikhov, K., Gut, M., Brun-Heath, I., Hubner, N.C., et al. (2013). Cell Stem Cell *13*, 360–369.

Jackson, M., Krassowska, A., Gilbert, N., Chevassut, T., Forrester, L., Ansell, J., and Ramsahoye, B. (2004). Mol. Cell. Biol. *24*, 8862–8871.

Jurkowska, R.Z., Jurkowski, T.P., and Jeltsch, A. (2011). ChemBioChem *12*, 206–222.

Liao, J., Karnik, R., Gu, H., Ziller, M.J., Clement, K., Tsankov, A.M., Akopian, V., Gifford, C.A., Donaghey, J., Galonska, C., et al. (2015). Nat. Genet., in press. Published online March 30, 2015. http://dx.doi.org/10.1038/ng.3258.

Ramsahoye, B.H., Biniszkiewicz, D., Lyko, F., Clark, V., Bird, A.P., and Jaenisch, R. (2000). Proc. Natl. Acad. Sci. USA *97*, 5237–5242.

Smith, Z.D., Chan, M.M., Mikkelsen, T.S., Gu, H., Gnirke, A., Regev, A., and Meissner, A. (2012). Nature *484*, 339–344.

Tsumura, A., Hayakawa, T., Kumaki, Y., Takebayashi, S., Sakaue, M., Matsuoka, C., Shimotohno, K., Ishikawa, F., Li, E., Ueda, H.R., et al. (2006). Genes Cells *11*, 805–814.