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STEM CELLS

A key to totipotency

> GATA2 is required... for MERVL expression and the expanded developmental potential of *mir-34a*-deficient pluripotent stem cells

Pluripotent stem cells — embryonic stem cells (ES cells, which are derived from the blastocyst inner cell mass (ICM)) and induced pluripotent stem cells (iPSCs; obtained through somatic reprogramming) — can give rise to all embryonic cell types, but rarely contribute to extra-embryonic cell lineages of the placenta and yolk sac. A study published in *Science* now reports that the microRNA (miRNA) *mir-34a* has a key role in this restriction of the developmental potential of mouse pluripotent stem cells.

The authors found that teratomas generated from mouse iPSCs and ES cells that are deficient in *mir-34a* (the most abundantly expressed of all *mir-34/449* miRNA family members in ES cells) not only contained derivatives of the three embryonic germ layers, but also had cellular and molecular features of extra-embryonic placental lineages. This increased developmental potential of *mir-34a*-deficient ES cells and iPSCs was also seen during embryoid body differentiation.

To confirm that *mir-34a*-deficient pluripotent stem cells have a greater developmental potential than wild-type cells *in vivo*, the authors injected four mouse ES cells into recipient morulae and traced the fate of their progeny in chimeric blastocysts. Whereas wild-type cells only gave rise to cells of the ICM, the progeny of *mir-34a*-deficient ES cells localized to both the ICM and the extra-embryonic trophectoderm in ~60% of chimeric blastocysts. An expanded cell fate potential of *mir-34a*-deficient ES cells was also seen in post-gastrulation embryos, although extra-embryonic lineages were generated with lower efficiency. Importantly, the authors showed that *mir-34a*-deficient ES cells have bidirectional cell fate potential at the single-cell level, rather than the two fates originating from a heterogeneous population of cells.

To investigate the molecular mechanisms underlying this bidirectional developmental potential, the authors compared the transcriptomes of wild-type and *mir-34a*-deficient iPSCs using RNA sequencing. Surprisingly, they found that the most highly expressed and differentially regulated transcripts in *mir-34a*-deficient iPSCs were those from the MERVL family of endogenous retroviruses (ERVs). Increased MERVL expression in *mir-34a*-deficient iPSCs and ES cells was confirmed by real-time PCR and MERVL-encoded Gag expression, and it was also confirmed to be associated with bidirectional cell fate potential, as this potential was lost upon MERVL silencing. Of note, expression of MERVL (shown by MERVL-Gag staining) and *Oct4* (a key pluripotency factor) were mutually exclusive, suggesting that *mir-34a*-deficient cells have a unique transcriptional state that is distinct from that of classic pluripotent stem cells.

So, how does *mir-34a* repress MERVL expression? Induction of MERVL loci in *mir-34a*-deficient ES cells was dependent on intact long terminal repeats (LTRs). More specifically, a minimal 250 bp fragment, $MERVL_{125–375}$, that contains a predicted TATA box was sufficient to drive transcription in *mir-34a*-deficient cells in a reporter assay. There was no sequence complementarity between miR-34a and $MERVL_{125–375}$, suggesting that repression does not occur through a direct RNA base-pairing mechanism. Instead, the authors found that the transcription factor GATA2 binds to the $MERVL_{125–375}$ region in *mir-34a*-deficient pluripotent stem cells and that GATA2 is required (but not sufficient) for MERVL expression and the expanded developmental potential of *mir-34a*-deficient pluripotent stem cells. Both GATA2 mRNA and protein levels were increased in *mir-34a*-deficient cells and reduced upon miR-34a overexpression, indicating that miR-34a directly represses GATA2. Given the ability of miRNAs to regulate multiple protein coding genes, it is possible that other miR-34a targets cooperate with GATA2 in regulating the cell fate potential of ES cells and iPSCs.

MERVL ERVs have been previously reported to be highly induced in totipotent two-cell (2C) blastomeres. The identification of *mir-34a* as a repressor of the bidirectional cell fate potential and of MERVL expression opens new avenues for the study of totipotency in cultured cells.

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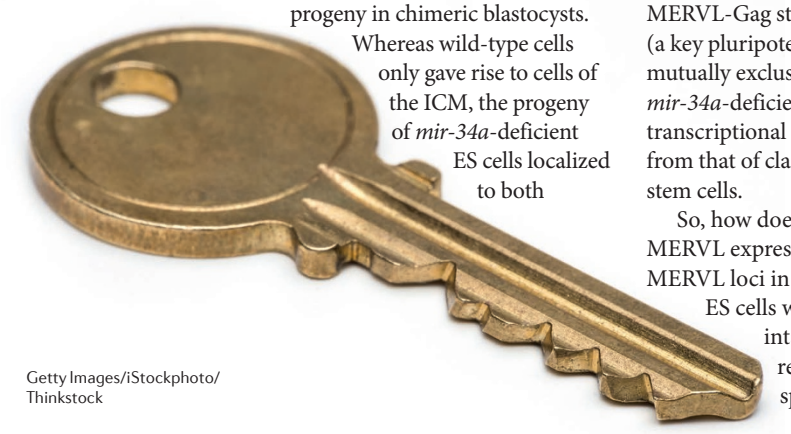

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ORIGINAL ARTICLE Choi, Y. J. *et al.* Deficiency of microRNA *mir-34a* expands cell fate potential in pluripotent stem cells. *Science* http://dx.doi.org/10.1126/science.aag1927 (2017)

FURTHER READING Smith, Z. D., Sindhu, C. & Meissner, A. Molecular features of cellular reprogramming and development. *Nat. Rev. Mol. Cell Biol.* **17**, 139–154 (2016) | Burton, A. & Torres-Padilla, M. E. Chromatin dynamics in the regulation of cell fate allocation during early embryogenesis. *Nat. Rev. Mol. Cell Biol.* **15**, 723–734 (2014)