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RESEARCH HIGHLIGHT

Moving towards totipotency without a single miR-acle

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Totipotency is the ability of a single cell to form an entire embryo, including extra-embryonic tissues, an ability we have yet to recapitalize, *in vitro*. In a recent paper published in *Science*, Choi *et al*. showed that pluripotent stem cells lacking microRNA miR-34a, have an expanded cell fate potential allowing differentiation into not only embryonic but also extraembryonic lineages.

When a sperm fertilizes an egg, a single-cell embryo termed a zygote is formed. The zygote, and its progeny in the first cell division, called the two-cell (2C) stage, are totipotent cells which are able to form the entire embryo and have an unrestricted developmental potential which allows differentiation into all embryonic and extraembryonic lineages. At later cell divisions of the pre-implantation embryo (4C, 8C, morula and blastocyst), cells start to lose totipotency. Developmental potential is gradually being restricted to inner cells forming the inner cell mass (ICM), which will generate the embryo, and to outer cells forming the trophectoderm (TE), which will generate extraembryonic tissues such as the placenta [1].

A unique hallmark of the murine totipotent 2C-stage embryo is transcriptional activation of the muERV-L (MERVL) family of endogenous retroviruses, which are assumed to play a role in transcriptional regulation of this stage, possibly by acting as alternative promoters to their proximal genes [2]. By using a reporter vector under the regulation of a MERVL element, it has been shown that within cultured ESC populations, 2C-like/bi-potential cells spontaneously arise at very low percentages [3]. These bi-potential cells resemble totipotent 2C-stage embryos in several key features: (i) activation of MERVL loci and MERVL-proximal genes, (ii) lack of core pluripotency protein expression (Oct4/Sox2/Nanog), (iii) an extended (or bi-potential) cell-fate potential allowing contribution to both embryonic and extraembryonic lineages. In addition, probing the activation of MERVL has been exploited to identify genetic manipulations that can induce bi-potential cells and increase their abundance in ESC cultures [3, 4].

In a recent study published in *Science*, Choi *et al*. [5] demonstrated that regulating the levels of miR-34a can produce bi-potential cells from murine pluripotent stem cell cultures, with a high efficiency. MicroRNAs are a group of small non-coding RNA molecules that regulate gene expression by post-transcriptional RNA silencing. In an earlier work, the same group has already shown that reducing the levels of miR-34a leads to an increase in efficiency of reprogramming to pluripotency [6]. Now, by combining several approaches, Choi *et al*. revealed that miR-34a$^{-/-}$ pluripotent stem cells have an extended cell-fate potential and can generate both embryonic and extraembryonic lineages. For both the teratoma and embryoid bodies (EBs) generated from miR-34a$^{-/-}$ pluripotent stem cells, marker levels of pluripotency derivative layers remained normal, implying that the cells kept their pluripotency, while a significant increase in key trophectodermal markers, such as Cdx2 and Elf5, was observed. Of note, these two key genes are also implicated in formation of other organs such as intestine [7] and mammary gland [8] and thus careful morphological examination showing evidence of trophectodermal cells present within the EBs, after 9 days of differentiation, was essential.

This extended cell-fate potential was evident also *in vivo*. When four GFP-labeled miR-34a$^{-/-}$ pluripotent stem cells were injected into a recipient morula, about 60% of the resulting blastocysts showed contribution to both ICM and TE, in comparison with WT pluripotent stem cell injection, which only contributed to ICM. This result was further validated with single miR-34a$^{-/-}$ pluripotent cell injection.

An RNA-seq comparison between miR-34a$^{-/-}$ and WT induced pluripotent stem cells (iPSCs) confirmed the correlation between bi-potential ESCs and MERVL activation [3, 4], where MERVL family transcripts were among the most highly and differentially expressed in miR-34a$^{-/-}$ iPSCs. Probing the expression of the MERVL-gag protein showed that miR-34a$^{-/-}$ pluripotent cultures are heterogeneous in relation to MERVL activation. Furthermore, MERVL-positive cells and Oct4-positive cells were mutually exclusive, further supporting a unique transcriptome for these cells.

Excitingly, the authors were further able to describe a detailed molecular mechanism linking miR-34a knockout to MERVL activation (Figure 1). By constructing several MERVL-regulated luciferase reporters, the authors showed that a minimal 250 bp fragment of MERVL's 5′ LTR (MERVL$_{125\text{-}375}$) was sufficient for strong induction in miR-34a$^{-/-}$ ESCs, and further predicted GATA-binding protein 2 (Gata2) tran-

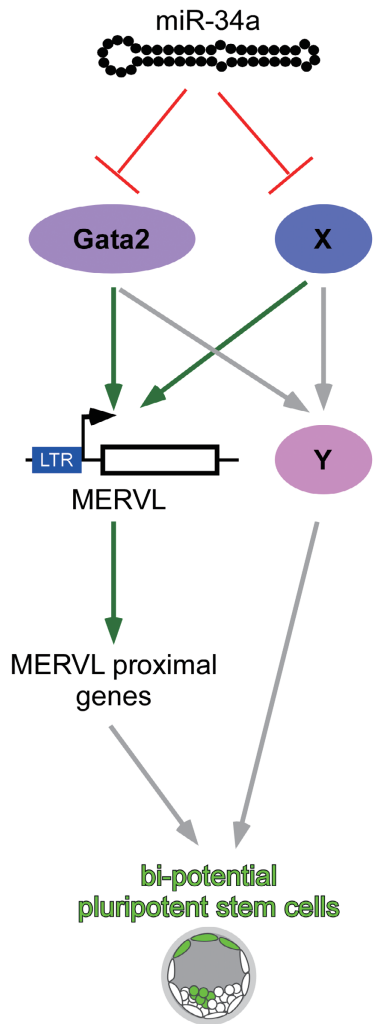

Figure 1 Molecular pathway describing how miR-34a possibly regulates induction of bi-potential ESCs. miR-34a directly inhibits the Gata2 transcription factor. Upon miR-34a deficiency, Gata2 binds and activates MERVL loci, with the aid of other factor/s inhibited by miR-34a ("X"). Activated MERVL loci act as alternative promoters to MERVL-proximal genes, which possibly regulate the bi-potential transcriptome. The extent to which MERVL activation plays a role in inducing the bi-potential state is currently unknown, and hence it is possible that other factors ("Y") work in parallel to mediate the bi-potential state, downstream of Gata2 and X. Red cross depicts inhibition, green arrow depicts induction and grey arrow stands for possible induction.

scription factor as the best possible candidate for regulating this MERVL fragment. Indeed, Gata2 was found to directly bind to the MERVL LTR, and knockdown of Gata2 in miR-34a$^{-/-}$ pluripotent stem cells abolished the induction of MERVL. An enrichment in transcription activating H3K27me3 may be involved in this process. Importantly, the authors showed that Gata2 harbors predicted miR-34a-binding sites and is likely to be a direct miR-34a target in pluripotent stem cells.

This study demonstrates a role of miR-34a deficiency in inducing bi-potential cells from pluripotent stem cells. It is the first study to describe a molecular mechanism of how MERVL loci are activated in induced bi-potential cells. Nevertheless, several interesting questions remain unanswered (Figure 1): is the activation of MERVL merely a marker for the extended cell-fate potential of 2C-stage embryos and bi-potential ESCs? Alternatively, does this activation play a mechanistic role in generating this unique transcriptome, possibly through MERVL-proximal gene activation? On this regard, does Gata2 contribute to a bi-potential phenotype via its positive effect on MERVL, or through another molecular mechanism? Moreover, in this study, Gata2 was shown to be a necessary but insufficient factor for MERVL induction in miR-34a-deficient cells, as its overexpression in WT ESCs was unable to activate MERVL. This raises the question of which other factor/s are repressed by miR-34a, which are important players in Gata2-mediated activation of MERVL (and in achieving a bi-potential phenotype) upon miR-34a de-repression. It has been shown that several gatekeepers such as Elf5 safeguard ESCs from transdifferentiating into trophoblast stem cells (TSCs, TE equivalent) by a mechanism involving methylation [9]. Thus, other intriguing questions are whether the methylation status of these gatekeepers is deregulated in miR-34a$^{-/-}$ pluripotent stem cells, and whether completely reprogrammed transdifferentiated-TSCs can be attained in miR-34a$^{-/-}$ pluripotent stem cells, without genetic manipulations. However, the present study provides us with considerable insight into the molecular barriers behind the first cell-fate decision and the mechanisms governing cell plasticity, characteristic of totipotency. Such understanding could later be translated into new therapeutic avenues.

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