Micro-managing and restraining pluripotent stem cells by MYC

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The MYC proto-oncogene, which encodes a master transcriptional regulator c-Myc (herein termed Myc), has a key role in stem cell (SC) pluripotency and tumorigenesis. Its deregulated expression contributes to cancer development and enhances the efficiency of reprogramming differentiated cells back to a pluripotent state in induced pluripotent SCs (iPSCs) that resemble embryonic SCs (ESCs). In addition to Myc's ability to directly regulate mRNA transcription, it also directly regulates microRNAs (miRNAs), thus providing an additional level of complexity to gene regulation. However, it had not been well understood how the complex network of Myc target genes contributes to pluripotency in ESCs and to tumorigenesis, two phenomena that are likely inter-related. In this issue of *The EMBO Journal*, Lin *et al* (2009) document a role of Myc in the maintenance of murine ESC (mESC) pluripotency through the regulation of a set of miRNAs that suppress differentiation, in addition to those that have been previously implicated in self-renewal capacity.

Myc is a bHLH transcription factor that dimerizes with Max to bind target DNA consensus sites to activate transcription. Myc also represses gene expression, in part through its interaction with Miz-1, but the general mechanism of

gene repression by Myc has been otherwise unknown (Eilers and Eisenman, 2008). Myc's transcriptional activity has been linked to tumorigenesis through target genes that are involved in cell cycle regulation, ribosomal biogenesis and metabolism; however, the role of Myc in the induction and maintenance of SC pluripotency is less well understood. Nonetheless, Myc is one of four transcription factors that are capable of reprogramming differentiated adult cells back to a pluripotent state (Takahashi and Yamanaka, 2006).

In addition to the induction of mRNA expression, Myc was documented to regulate miRNAs (O'Donnell *et al*, 2005). As Myc is documented to have a role in the maintenance or induction of the pluripotent SC state, it stands to reason that miRNA targets of Myc in ESCs would have a role in pluripotency and self-renewal (Singh and Dalton, 2009). In this regard, Lin *et al* sought to understand the role of Myc in regulating miRNAs and in the maintenance of the SC state. By using a primer extension PCR assay for 192 selected miRNAs, Lin *et al* determined the expression profiles of these miRNAs in mESCs and their derivatives. This panel of cells allowed for the stringent identification of key miRs, the expression levels of which are correlated with MYC status. The authors found

Figure 1 Schematic depicting known pluripotent stem cell factors, with Myc regulating miRNAs involved in self-renewal and those reported by Lin *et al* involved in repression of differentiation, as well as mRNAs, such as ones involved in mitochondrial function and DNA metabolism among others.

that expressions of the oncogenic miRNA cluster miR-17-92 and the related miR-302 were induced by Myc in ESCs. These miRNAs are enriched in iPSCs and ESCs as compared with fibroblasts (Laurent *et al*, 2008; Chin *et al*, 2009; Wilson *et al*, 2009). It is notable that the miR-290 family, which shares consensus seed sequence with miR-302, has been documented to be up-regulated by Myc and to have a role in G1-S transition in pluripotent SCs, suggesting that this entire group of related miRNAs participates in SC renewal (Wang and Belloch, 2009) (Figure 1).

Lin *et al* focused on a set of miRNAs, including miR-141, miR-200, miR-338 and miR-429, which were identified to behave as direct Myc targets. Ectopic expression of this group of miRNAs collectively retarded the differentiation of ESCs after removal of LIF. Conversely, locked nucleic acid anti-miRs facilitated differentiation. The authors then determined the putative target mRNAs of these miRNAs in ESCs by using synthetic miRNA duplexes. Decreased expression of mRNAs, which were repressed by miRNA duplexes and also by ectopic Myc, provided the signature of the miRNA targets. Among the putative targets identified are mRNAs involved in differentiation, such as cadherin 11, TGF β RIII, Foxf2, Semaphorin and Neuropilin (Figure 1). These observations suggest that one function of Myc in SC maintenance is its repression of differentiation through the induction of specific

miRNAs. This perspective is indeed a new one, particularly as expression of miRNAs generally increases with cellular differentiation, such that different tissue types have distinct characteristic miRNA profiles. Furthermore, the identification of these miRNAs provides a clue to an additional mechanism, besides its known interaction with the Miz-1 transcription factor, of how Myc mediates gene repression.

Collectively with other studies, the work by Lin *et al* contributes to an emerging picture of a role for Myc in the induction and maintenance of the pluripotent SC state, through the regulation of specific mRNAs by direct transcriptional activation, as well as by modulation of mRNA levels by miRNAs induced by Myc. A putative core SC mRNA signature induced by Myc involves mitochondrial function and DNA metabolism (Wong *et al*, 2008), whereas miRNAs regulated by Myc are involved in cell cycle regulation, SC renewal and suppression of differentiation. The lessons learned here are likely to provide additional insights into the role of Myc in tumorigenesis, particularly with the emergence of the cancer SC concept.

Conflict of interest

The author declares that he has no conflict of interest.

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