Micro-RNAs meet epigenetics to make for better brains

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Recent studies have highlighted the importance of regulatory non-coding RNAs and epigenetics in controlling the differentiation of somatic stem cells. Two major pathways characterize these fields: micro-RNAs (miRNAs) and DNA methylation. In this issue of *EMBO Reports*, Lv *et al* show that during mammalian corticogenesis, miR-15b inhibits cytosine demethylation by targeting Tet3, a key methylcytosine dioxygenase. This leads to the epigenetic downregulation of cyclin D1. As a result, cell cycle and differentiation of neural progenitors are altered, promoting their switch to neurogenesis. Hence, Lv *et al* elegantly bring together miRNAs and DNA methylation in the cell cycle control of neural progenitors and neurogenesis.

See also: X Lv *et al* (December 2014)

The correct switch from proliferation to differentiation of neural stem cells during corticogenesis is essential for normal development, and severe diseases may arise from an imbalance of this process. Regulatory non-coding RNAs and epigenetic modifications are key players underlying this switch, among which regulation of gene expression by miRNAs and DNA methylation are prominent mechanisms.

miRNAs are short oligonucleotides of about 22 bases that inhibit translation of mRNAs through activation of the RNA-induced silencing complex. Either by triggering the degradation of their targets or by suppressing their translation, miRNAs can regulate the levels of several proteins at the same time. For this reason, miRNAs are considered as ideal regulators of complex gene networks and interfering with their biogenesis or expression has been shown to influence brain development [1].

In parallel to regulatory non-coding RNAs, epigenetic mechanisms also control stem cell differentiation. The classical and most studied epigenetic modifications are on histones and DNA [2], the latter by methylation on position 5 of cytosine (5mC), which is considered as an additional base of the genome. Accumulation of 5mC at transcription factor binding sites and promoter regions negatively correlates with gene expression while, conversely, enrichment of 5mC within the gene body has been linked to up-regulation of transcription [2]. Despite 5mC being one of the most studied epigenetic marks, an active DNA demethylation mechanism that could explain the dynamic patterns of 5mC in the absence of cell division has been described only recently. Members of the Ten-eleven translocation (Tet) family oxidize 5mC to 5-hydroxymethylcytosine (5hmC), which is the starting point of an oxidation cascade resulting in the replacement of the modified pyrimidine by a new cytosine through DNA repair mechanisms [2]. Among the oxidative products, 5hmC is highly abundant in the nervous system where it seems to contribute to promoter poising, which is crucial for rapid transcriptional activation during stem cell commitment and potentially explains the function of DNA hydroxymethylation during development and adulthood [3].

miRNAs and epigenetics are both known to regulate corticogenesis, and it has long been recognized that epigenetic marks influence miRNA expression and that miRNAs control DNA methylation [4]. Conversely, cases of miRNAs that control the reverse process, DNA demethylation, have been reported only recently and in few tissues [5–7].

In this issue of *EMBO Reports*, Lv *et al* [8] extend these findings by identifying the first miRNA controlling DNA demethylation

in the developing mammalian brain. Specifically, the authors looked at the expression of miRNAs during corticogenesis focussing their attention on miR-15b. This miRNA was detected in both germinal and neuronal layers with a peak of expression that coincided with the peak of neurogenesis. Intrigued by this pattern, the authors characterized the effects triggered by manipulating miR-15b expression. They find that its overexpression increased the proportion of neurons in the cortical plate while its downregulation had the opposite effect. These changes in cell distribution throughout the cortex were due to an altered migration of newborn neurons and, in addition, to a different behavior of neural progenitors as evidenced by a change in the proportion of apical, radial glial cells and basal, intermediate progenitors undergoing S phase or mitosis. Despite the fact that the length of the cell cycle was not assessed in this study, the decreased proliferation rate and increased neurogenesis upon miR-15b overexpression could potentially be explained by miR-15b causing a lengthening of the cell cycle, which promotes differentiation [9].

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The study could have ended here by adding miR-15b to the long list of miRNAs with a function in brain development without knowing how this function is actually executed. Instead, here, the authors switch gears and move to the next level of analysis.

Using bioinformatic tools, Lv *et al* identified four miR-15b seed sequences in the *Tet3* mRNA and demonstrated their functional relevance by luciferase assays. In particular, gain and loss of function of miR-15b were not only found to consistently alter the levels of *Tet3* but also those of 5hmC, thus directly linking this miRNA to epigenetic modifications. As the next intuitive step, the authors aimed to identify at least one gene whose epigenetic regulation by miR-15b/*Tet3* could be held responsible for the observed changes in corticogenesis. Perhaps inspired by a recent study linking this miRNA to cyclin D1 expression in gliomas [10], the authors investigated the effect of *Tet3* expression on this key regulator of the G1 phase of the cell cycle. Cyclin D1 has been linked to neurogenesis before and is therefore a promising candidate for controlling the cell cycle and differentiation of neural progenitor cells during corticogenesis [9].

Manipulation of *Tet3* in cultured cells positively correlated with *cyclin D1* expression. In addition, knockdown of *Tet3* or overexpression of miR-15b both decreased the levels of 5hmC relative to 5mC at the *cyclin D1* locus. This correlated with a decrease in *cyclin D1* mRNA levels; however, this result must be taken with caution since the *cyclin D1* transcript has also been reported to be targeted directly by miR-15b [10]. Importantly, the authors could show that the manipulation of *Tet3* alone regulated neural progenitor cell proliferation *in vivo* and that *Tet3* overexpression partially rescued the neural progenitor proliferation and neurogenesis defects upon miR-15b overexpression, thus strengthening the relationship between miR-15b and *Tet3* beyond the direct targeting of *cyclin D1* by miR-15b [10].

Given that miR-15b appears to regulate both *cyclin D1* [10] and *Tet3* [8] mRNAs, we can speculate on the existence of a synergistic action by which the short-term effect of miR-15b is to downregulate the translation of the *cyclin D1* mRNA while, concomitantly, it induces a long-term epigenetic repression of the *cyclin D1* locus via downregulation of *Tet3*. Certainly, this elegant link between a miRNA, epigenetics, and stem cell differentiation is not expected to solely rely on *cyclin D1*. Many more loci might undergo changes in 5hmC as a result of miR-15b/*Tet3* activity, but these were not identified by Lv *et al*. In this context, we speculate that targets of miRNAs/Tets may be cell specific with certain loci being hydroxymethylated

Figure 1. Stem Cell Race.

Tet3 is depicted riding a motor-cyclin model *D1* equipped with new 5mC/5hmC-technology wheels. MiR-15b marks the way to the pit stops of this reckless circuit.

in apical, radial glial cells, and others being modified by the same players in different cell types, such as basal, intermediate progenitors or neurons, which could potentially explain the additional effects observed on neuronal migration upon manipulation of miR-15b [8]. Assessing specific epigenetic changes in individual cell types rather than whole organs represents the next challenge for the field. For now, Lv *et al* extended recent reports on the regulation of Tet family members by miRNAs [5–7] and their roles in brain development [3]. More importantly, this study has the merit to elegantly link key areas of investigation by encompassing the study of miRNAs, epigenetics, cell cycle progression, and differentiation of neural progenitors (Fig 1). We find it remarkable that such a link is provided through cyclin D1, a gene extensively studied during corticogenesis [9] and now also a target of miR-dependent epigenetic modification during brain development.

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