[Cell Adhesion & Migration 3:2, 141-142; April/May/June 2008]; ©2008 Landes Bioscience

Journal Club

REST regulation of neural development

From outside-in?

Bor Luen Tang

Department of Biochemistry; Yong Loo Lin School of Medicine; National University of Singapore; Singapore, Singapore

Key words: laminin, neural differentiation, neuron, neural stem cells, REST

The RE1 Silencing Transcription Factor (REST) is a master regulator of neuronal and glial fate specification that acts at multiple levels of stem cell differentiation, through the engagements of a plethora of cofactors and histone modifying proteins. Buckley and colleagues now show that low levels of REST are required even after the transition from embryonic stem cells to more committed neural progenitors, as well as neurogenesis. Generations of nestin-positive, followed by βIII-tubulin-positive as well as MAP2-positive phenotype are impeded by REST ablation, and REST-depleted neural stem cells are defective in adherence, migration and survival. These defects can be rescued by exogenous laminin. The findings shed new light on a previously underemphasized aspect of REST function, namely extracellular matrix regulation during neural differentiation.

Introduction

Establishment and maintenance of the neuronal phenotype entail selective expression of neuron-specific genes, which transcription are normally repressed in non-neuronal cells. The Repressor Element 1(RE1) found in the promoter of a myriad of neuronal genes is a key cis-regulatory element for such a repression. RE1 binds the zinc-finger transcription repressor RE1 Silencing Transcription factor (REST)[1] (also known as Neuron-Restrictive Silencer Factor (NRSF).[2] REST expression is high in blastocysts and embryonic stem (ES) cells, but diminishes with the onset of neural differentiation. Downregulation of REST appears to be an essential for neural differentiation. However, it is now clear that REST is not simply a transcriptional repressor. By engaging a multitude of cofactors and histone modifying enzymes, REST regulates the expression of a large network of neuronal genes through epigenetic suppression as well as activation of transcription.[4] A recent study had revealed both canonical and non-canonical RE1 sites at over 2,000 genomic loci, with REST recruitment to a developmentally-independent set of genes found in cells at all stages of neural development, as well as an ES cell-specific subset of target genes.[5] Transcriptional dysregulation associated with REST has been shown to be involved in the pathology of neurodegenerative and neurological diseases,[6] as well as malignancies.[7]

Correspondence to: Bor Luen Tang; Department of Biochemistry; Yong Loo Lin School of Medicine; National University of Singapore; 8 Medical Drive; Singapore 117597, Singapore; Tel.: +65.6516.1040; Fax: +65.6779.1453; Email: bchtbl@nus.edu.sg

Submitted: 1/22/09; Accepted: 2/24/09

Previously published online as a *Cell Adhesion & Migration* E-publication: www.landesbioscience.com/journals/celladhesion/article/8278

A sustained low level of REST is important for neural differentiation and neurogenesis. Analysis of REST function and the effects of REST depletion during neural development in vivo are understandably difficult in view of the fact that Rest -/- mice die at around E11.5. Buckley and co-workers had developed in vitro models of ES cell differentiation in which a combination of targeted deletion and small hairpin (sh) RNA-mediated silencing allowed the authors to generate ES cell lines with REST transcript levels at an equivalent (REST-100), 50% (REST-KD/50), or 1% of wild type levels.[8] The last of which, with undetectable levels of REST protein, are functionally designated as REST-null cells. These ES lines were then differentiated in culture and monitored at stages which the authors roughly defined as neural stem cells (NSCs, days 2–8, expressing the markers Sox1 and nestin), neural progenitor cells (NPCs, days 8–12, expressing the markers Ngn1 and Mash1) and neurons (days 10–18, expressing βIII-tubulin, Tau, MAP2 and other neuronal markers). While ablation of 50% REST had no discernible effect on ES cell neural differentiation, ablation of REST expression to 1% of wild type levels appeared to impede the development of NSCs, NPCs and neurons. At the earlier times, a lack of nestin-positive cells was noticeable. At later time points, it was clear that neurogenesis, as indicated by neuronal marker expression, was also affected.

These results, though not exactly surprising, reinforces a more contemporary notion that the role of REST in neural differentiation is complex, and that it does not work like a simple on-off repressor switch. In fact, the neuronal marker genes that are known to be repressed by REST did not become precociously expressed in REST-ablated cells as one might expect. The data instead suggest that a sustained low level of REST is essential for neural differentiation and neurogenesis. To rule out off-target effects of their sh-RNA, the authors attempted to rescue the phenotypes with overexpression of REST. It turns out that REST expressed at 8% of wild type levels (in a REST-null + REST line) largely reversed the differentiation phenotype exhibited by the REST-null cells.

The authors' observation that ES cells with only 50% wild type REST levels behaves like wild type ES cells is, however, in contrast to finding presented in another recent report. Singh et al. showed that heterozygous Rest deletion or Rest silencing impairs ES cells' pluripotency and capacity for self-renewal.[9] This phenotype was not

observed by the authors, who also noted insignificant changes to the levels of the stemness genes Nanog and Oct4 in their ES cells with 50% wild type REST levels. Reasons for the discrepancy in observations between the two groups are unclear. However, as Rest +/- ES cells could be used to generate mice with germ-line transmission of the genotype, heterozygocity for Rest could not have affected in vivo development.

REST regulation of ECM laminin expression may underlie the neural differentiation phenotypes. Another important finding of the paper pertains to how neural differentiation and neurogenesis was impeded by loss of REST. The authors observed that REST-null cells are defective in adhesion and migration, phenotypes that became particularly pronounce on glass surfaces, and which are at least partially rescued by the expression of REST at 8% of wild type levels. Reasoning that dysregulation of extracellular matrix (ECM) components or cell adhesion molecules may underlie the loss of cell adhesion and migration observed, the authors plated their cells on precoated ECM components and check for possible reversal of phenotype. Interestingly, the ECM component laminin not only enhanced cell adhesion and survival, but significantly reversed the neural differentiation and neurogenesis phenotype of REST-null cells. This activity of laminin is not down to augmentation of cell adhesion alone, as fibronectin is far less effective in this regard, while poly-D-lysine had no enhancement effect at all. The authors examined the expression pattern of some laminin subunits, and noted that these are generally highly expressed in ES cells. Their expression levels drop during NSC differentiation, but gradually increase again during neurogenesis. REST-null cells have significant lower levels of these laminin subunits throughout neurogenesis, and expression is partially restored by increasing REST levels to 8% of wild type levels in the REST-null + REST cells. As genes encoding laminin αsubunits are direct REST targets,[11] it appears that severe REST depletion could impair neural differentiation and neurogenesis, owing at least partially from a loss of laminin secretion from the cells.

The above findings brought to light a particular aspect of REST activity that is worth noting, even in an arguably limited in vitro experimental paradigm. Although REST levels are roughly inversely related to that of laminin during neural differentiation of ES cells, a total loss of REST appears to significantly reduce laminin subunits expressions during neurogenesis. A low but sustained level of REST throughout neural differentiation is therefore important in this regard. It would be of interest to understand how low levels of REST function in sustaining the transcription of genes like laminin subunits, which it represses at wild type levels. It may be somewhat unexpected that of the myriad of genes that are potentially affected by a severe depletion of REST, that a single ECM component would be critical for differentiation of nestin-positive NSCs and subsequent neurogenesis. Laminins have multiple known roles in neural stem cell niches and is required for proliferation, differentiation and survival of NSCs and neurons.[12,13] Like many other ECM components, laminin engages cell surface integrins in outside-in signaling processes that regulate growth and survival of multiple cell types in different tissues. In stem cell niches in vivo, laminin could be secreted by multiple cell types, and regulate NSC differentiation in both an autocrine or paracrine manner.

It should be noted that REST regulates the expression of a good number of cell surface molecules with adhesion and migratory functions.[5,10–12] It would therefore be of interest to examine if some of these may also be important in NSC differentiation and neurogensis. In any case, understanding how REST regulate laminin expression in stem cell niches, and how these would affect cell adhesion and migration, would appear to be an important aspect of any attempt to promote endogenous neurogenesis for therapeutic purposes.

References

1. Chong JA, Tapia-Ramírez J, Kim S, Toledo-Aral JJ, Zheng Y, Boutros MC, et al. REST: A mammalian silencer protein that restricts sodium channel gene expression to neurons. Cell 1995; 80:949–57.
2. Schoenherr CJ, Anderson DJ. The neuron-restrictive silencer factor (NRSF): A coordinate repressor of multiple neuron-specific genes. Science 1995; 267:1360–63.
3. Ballas N, Grunseich C, Lu DD, Speh JC, Mandel G. REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. Cell 2005; 121:645-57.
4. Ballas N, Mandel G. The many faces of REST oversee epigenetic programming of neuronal genes. Curr Opin Neurobiol 2005; 15:500-6.
5. Johnson R, Teh C, Kunarso G, Yew Wong K, Srinivasan G, Cooper ML, et al. REST regulates distinct transcriptional networks in embryonic and neural stem cells. PLoS Biol 2008; 6:256.
6. Ooi L, Wood IC. Chromatin crosstalk in development and disease: Lessons from REST. Nat Rev Genet 2007; 8:544–54.
7. Westbrook TF, Martin ES, Schlabach MR, Leng Y, Liang AC, Feng B, et al. A genetic screen for candidate tumor suppressors identifies REST. Cell 2005; 121:37–848.
8. Sun YM, Cooper M, Finch S, Lin HH, Chen ZF, Williams BP, et al. Rest-mediated regulation of extracellular matrix is crucial for neural development. PLoS ONE. 2008; 3:3656.
9. Singh SK, Kagalwala MN, Parker-Thornburg J, Adams H, Majumder S. REST maintains self-renewal and pluripotency of embryonic stem cells. Nature 2008; 453:223-7.
10. Sun YM, Greenway DJ, Johnson R, Street M, Belyaev ND, Deuchars J, et al. Distinct profiles of REST interactions with its target genes at different stages of neuronal development. Mol Biol Cell 2005; 16:5630-8.
11. Otto SJ, McCorkle SR, Hover J, Conaco C, Han JJ, Impey S, et al. A new binding motif for the transcriptional repressor REST uncovers large gene networks devoted to neuronal functions. J Neurosci 2007; 27:6729-39.
12. Colognato H, ffrench-Constant C, Feltri ML. Human diseases reveal novel roles for neural laminins. Trends Neurosci 2005; 28:480-6.
13. Shen Q, Wang Y, Kokovay E, Lin G, Chuang SM, Goderie SK, et al. Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. Cell Stem Cell 2008; 3:289-300.