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Different Manners of Interplay between MicroRNAs and Gene Programs in Neuronal Specification

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Abstract

MicroRNA (miRNA) plays a critical role in self-renewal and differentiation of neural stem cells as well as proliferation and specification of neuronal progenitors through regulating spatial and/or temporal expression of its targets. It's well documented that mutually exclusive or opposing gradient expression pattern of miRNA and its target gene diversifies subtypes of neurons and even specifies distinct functional properties of bilaterally symmetric neurons. Unlike these mechanisms, we recently showed that miR-183-96-182 cluster timely differently shuts off co-expressed transcription factor SHOX2 in the progenitor pool to generate two subtypes of low threshold mechanoreceptor (LTMR) neurons, early close of SHOX2 expression promoting the fate of Aβ slowly adapting (SA) LTMR neurons and late off leading to the identity of Aδ LTMR neurons. It indicates that population sizes of these two LTMR neurons are reversely generated depending on variant abundance of miR-183 cluster in dorsal root ganglion (DRG). This new mechanism of precisely controlling off-time of key specification gene expression by co-expressed miRNA to regulate both the fates and population sizes of subtypes of neurons broads our understanding of how diverse neurons derived from a same progenitor pool are specified by miRNA-regulated gene program, and will potentially help us to efficiently differentiate human iPS cells to one certain type of cells for therapeutics purpose or making drug-screening models. In this commentary, we discuss our recent findings in context of how miRNA interplays with gene programs in four different manners to specify neuronal fates, and propose future directions.

Keywords: MiR-183; MiR-96; MiR-182; Population size; Specification; Fate switch; Shox2; Timing control

Mechanisms Used by MicroRNAs to Specify Neuronal Progenitors

MiRNA plays a vital role in differentiation of neural stem cells and specification of neuronal progenitors through regulating spatial and/or temporal expression of gene programs involved in above developmental events. Somatosensory neurons in DRG encompass over ten neuronal subtypes demonstrated by single cell sequencing data [1,2], how all these types of sensory neurons came from the same progenitor pool derived from trunk neural crest are diversified remains unclear. Our recent findings showed that miR-183-96-182 cluster (miR-183, miR-96 and miR-182, hereafter referred to miR-183 cluster) reversely regulates the fates and population sizes of Aβ SA-LTMR neurons ($TRKC^+/Runx3^-$; a class of heavily myelinated sensory neurons with rapid axonal conduction velocities ranging from 16-100 m/s [3]) and Aδ LTMR neurons ($TRKB^+/NECAB2^+$; a class of lightly myelinated sensory neurons with intermediate axonal conduction velocities ranging from 5-30 m/s [3]) in the developing of DRG sensory neurons by timely different turning off subtype specification gene program including vital transcription factor Shox2 in the progenitors [4]. SHOX2 protein is expressed in all hybrid $TRKC^+/TRKB^+$ DRG neurons at E10.5 in wild type mice and is rapidly turned off in ~75% and almost all $TRKC^+$ neurons at E11.5 and E12.5, respectively. In contrast, SHOX2 expression is retained in ~75% and 25% $TRKB^+$ neurons at E11.5 and E12.5, respectively [4]. It's previous reported that conditional knock out of Shox2 in mouse DRG resulted in decrease of $TRKB^+$ LTMR neurons and increase of $TRKC^+$ neurons because Shox2 promotes TRKB and represses TRKC [5,6], but how Shox2 is terminated in $TRKC^+/TRKB^+$ progenitors in order to stop the genesis of Aδ LTMR neurons in favor of $TRKC^+$ neurons was unknown. We recently found that *Wnt1-Cre; miR-183-96-182* $^{flox/flox}$ mice in which miR-183 cluster was ablated in DRG neurons derived from Wnt1-expressing trunk neural crest cell fail to extinguish SHOX2 expression in DRG by E11.5 stage, however, have down regulated level of SHOX2 similar to control mice by E12.5. This extension of SHOX2 expression before E12.5 consequently leads to an increase in proportion of $TRKB^+/NECAB2^+$ Aδ LTMR neurons at expense of $TRKC^+/Runx3^-$ Aβ SA-LTMR neurons, while no affects with the total number of DRG neurons [4]. Further gain-of-function and loss-of-function experiments showed that overexpression of miR-183 cluster in chicken DRG, or depletion of Shox2 in *Wnt1-Cre; Shox2* $^{flox/flox}$ mice, both increase Aβ SA-LTMRs population at expensive of Aδ LTMRs. Therefore, combining with the *in situ* hybridization data showing that miR-182 is ubiquitously expressed in all DRG neurons rather than cell-type specific pattern from E10.5 to E12.5, our data demonstrated that co-expressed miR-183 cluster shuts off SHOX2 expression in partial of $TRKC^+/TRKB^+$ progenitors earlier than the others to initiate the alternative fate of Aβ SA-LTMRs instead of continuously driving progenitors to the fate of Aδ LTMRs. The way miR-183 cluster participates in the specification of LTRMs is to determine the off-time of SHOX2 expression in a progenitor pool

rather than establishing selective expression pattern in partial progenitors. This new manner by which miRNA specifies neuronal fates belongs to temporal expression control model and is characterized by ubiquitously co-expressed miRNA in progenitors controlling off-time of early specification gene programs to favour the late one. We name this model as "expression off-time control manner".

Another temporal expression control model by which miRNA works with specification gene programs is "expression onset-time control manner", which is featured by ubiquitously co-expressed miRNA in progenitors controlling onset-time of late specification gene program to switch neuronal fates. The well example of this manner is that four miRNAs including miR-129, miR-155, miR-214 and miR-222, highly expressed in early retina progenitors inhibit translation of Xotx2 and Xvsx1 and down-regulation of these miRNAs in late stage allows onset of protein translation of Xotx2 and Xvsx1 which determine the fate of late-born bipolar neurons [7]. In addition to temporal expression control manners, there are two well documented spatial expression control models. One is "expression boundary control manner" which is featured by mutually exclusive or opposing gradient expression pattern of miRNA and its target gene to generate and maintain a clear boundary between two subtypes of neurons. For example: segmental expression of *Hox* genes regulated by microRNAs along rostro-caudal axis contributes to specification of subtypes of motor neurons in the spinal cord. MiR-27 (including a/b family members) induced by Hoxc8 is enriched in Hoxc8 domain and lower in Hoxa5 domain to generate Hoxa5/Hoxc8 boundary between rostro brachial and caudal brachial spinal region by repressing transcription noise of Hoxa5 in Hoxc8 motor neurons [8]. Similarly miR-196 is involved in specification of lumbar motor neurons by restricting the caudal extent of Hoxb8 expression to the thoracic-lumbar intersect [9]. Whereas during specification of medial-lateral motor neurons in the spinal cord, higher miR-9 level in late-born motor neurons restricts its target OC1 expression only in early-born neuron to allow genesis of late-born motor neuron from a same progenitor pool [10]. During specification of dorsal-ventral neurons in spinal cord opposing gradient expression of miR-17-3p and its target Oligo2 in $Irx3^+$ V2 interneurons and $Olig2^+$motor neurons constructs $Irx3^+/Olig2^+$ boundary, and genetic depletion of miR-17-3p leads to an increase of motor neurons at expense of V2 interneurons [11]. Moreover, two pairs of miRNAs and its target transcription factors are mutually exclusive expressed in bilaterally symmetric ASE chemosensory in nematode *C. elegans* and specify lateralized function of ASE chemosensory into ASER (right) and ASEL (left). High miRNA lys-6 level in ASEL represses ASER-promoting cog-1 expression and cog-1-induced miR-273 is exclusively expressed in ASER to repress die-1 which drives ASEL fate and induces lys-6 expression [12-14]. The other spatial expression control model is "expression gradient control manner", which is characterized by opposing gradient expression of miRNA and its target mRNA generating a gradient of zero-intermediate-high level of target protein expression. MiR-7 is expressed in a Pax6-opposing ventral-dorsal gradient in the forebrain ventricular wall to build a gradient of zero-intermediate-high level of PAX6 protein from ventro-dorsal which is essential for the generation of the correct quantity of dopaminergic neurons in the olfactory bulb [15]. No matter by which manner miRNA interplays with specification gene program, the basic molecular mechanism is that proper level of miRNA represses the protein expression of target gene to a designated threshold level in a right place during a correct period.

Switch Role of MiRNA and Potential Application

It's shown that the output of repression of target gene expression (switching off or fine-tuning) by miRNA depends on the abundance of both mRNA and miRNA as well as the number and affinity of miRNA binding sites on mRNA 3' UTR [16]. Thus, the increase of expression level of all three members of miR-183 cluster from E10.5 to E12.5 (unpublished data Peng et al.) and two conserved miR-183 cluster binding sites in Shox2 3' UTR are believed to contribute to rapid extinction of SHOX2 expression around E11.5 by miR-183 cluster which is needed for generating proper Aβ SA-LTMR neurons in DRG. Similarly switching off targets' expression happened on miR-200 family which encompasses 5 members sharing related seed sequence. Induced expression of miR-200 by E2F3 can rapidly switch off E2F3 protein level through 5 conserved binding sites on E2F3 mRNA in late S phase to allow cell enter G2 phase [17] and during mesenchymal-epithelial transition miR-200 can also rapidly extinguish the expression of Zeb2 which bears 5 or 6 conserved miR-200 binding sites [18,19]. So, the strategy of a miRNA family controlling temporal expression of target genes carrying multiple binding sites can be used for differentiating human iPS cells to one certain type of cells for therapeutics purpose or for making drug screening model. For example, blocking miR-132 with antisense oligonucleotide increases Nurr1 expression and then promotes mouse ES cells to differentiate into midbrain dopaminergic neurons which are lost in patient with Parkinson's disease [20], and therefore transplantation of these dopaminergic neurons is supposed to be able to treat Parkinson's disease. Since it's known that $TRKB^+$/$NECAB2^+$ Aδ LTMR neurons are involved in neuropathic pain [21], knocking down miR-183 cluster by antagomir or knock out miR-183 cluster and/or SHOX2 3' UTR containing miR-183 binding sites would increase the efficiency of differentiating human iPS cells into $TRKB^+$/$NECAB2^+$ Aδ LTMR neurons to establish a neuropathic pain drug screening model. Since expression of Shox2 and miR-183 cluster is in majority of or all DRG neurons, respectively, it's speculated that they may also participate in specification of other subtypes of DRG sensory neurons. So further efforts are needed to investigate if loss of miR-183 influences subtypes of neurons within TRKA population. In addition, it's interesting to explore if other highly expressed miRNAs in developing DRG are also involved in diversification of sensory neurons in a similar way to miR-183 cluster does.

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