Biochemical and Biophysical Research Communications 419 (2012) 27–31

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

NeuroD1 is an upstream regulator of NSCL1

Woo-Young Kim *

Department of Molecular, Cellular and Developmental Biology, University of Colorado, CO 80309, USA
College of Pharmacy, Sookmyung Women's University, Seoul 140-742, Republic of Korea

ARTICLE INFO

Article history:
Received 12 January 2012
Available online 28 January 2012

Keywords:
NeuroD1
NeuroD2l
NSCL1
NSCL2
Cerebellum development

ABSTRACT

Cell fate determination and differentiation during neurogenesis and myogenesis involve the sequential expression of several basic helix-loop-helix (bHLH) transcription factors. The expression of NeuroD1/2 and the expression of NSCL(Nhlh)1/2 are closely related in many developing peripheral and central neuronal cells, suggesting an epistatic relationship between these two bHLH transcription factor families during neurogenesis. To investigate this relationship, a murine neuroblastoma cell culture system and single/double knock-out (KO) mice of NeuroD1 and NeuroD2 were utilized for the gain-of-function and loss-of-function approaches, respectively. Both NeuroD1 and NeuroD2 were able to induce the transcription of NSCL1 in vitro; however, they were not able to activate NSCL2 transcription. The DNA-binding ability of NeuroD1 was essential for NSCL1 induction. To examine the epistatic relationship in vivo, we examined the expression of NSCL1 and NSCL2 in NeuroD1 and NeuroD2 KO mice and NeuroD1/2 compound KO mice by in situ hybridization, RT-PCR and Northern blotting. The expression of NSCL1 was lower in the NeuroD1 KO mice and was not further decreased in the double KO mice. However, the expression of NSCL2 did not change in either the single KO or double KO mice. These results demonstrate that NeuroD1 is an upstream regulator of the NSCL1 gene but not the NSCL2 gene in mice. In addition, NeuroD2 is not involved in this regulatory pathway in vivo.

1. Introduction

Basic helix-loop-helix (bHLH) proteins comprise a large family of transcription factors that are involved in cell fate determination and differentiation during development in many organisms. These proteins are defined by the presence of a basic domain that binds a specific DNA sequence known as the E-box (GANNTG) and an HLH domain that promotes dimerization [1]. Murine neuronal development is regulated by the orchestrated expression of several of these bHLH transcription factors. The activating bHLH transcription factors, including the Neurogenin, Math, and NeuroD families, play a central role in the development of the mammalian central neural system (CNS). Based on numerous expression studies and gene deletion experiments in mice, these bHLH proteins have been divided into two groups based on the onset of their expression and their function during neurogenesis. The first/early group includes Neurogenin, which is expressed in proliferating neuronal precursors and is believed to regulate cell fate determination [2–5]. The second/late group, which includes NeuroD, is mainly expressed during the terminal stages of neurogenesis and regulates cell survival and maturation [6–8]. These bHLH transcription factors occasionally show epistatic relationships. For example, Neurogenins function upstream of NeuroD1 in murine neurogenesis. In mice, many of the neurons that express NeuroD1 are derived from Neurogenin-expressing neural precursors. Ngn1 and Ngn2 are expressed during the early stage of the development of cranial sensory ganglia precursors and the dorsal root ganglia precursor cells which differentiate later into neurons that express NeuroD1. More conclusively, studies using the Neurogenin mutant mice and NeuroD mutant mice demonstrated the sequential relationship of these two transcription factor. Neurogenein-1 is essential for the generation and maintenance of the progenitor cells that give rise to the inner ear vestibular-cochlear ganglia (VCG), as demonstrated by the complete absence of the VCG in Neurogenin-1-null mice [9]. NeuroD1 is expressed in postmitotic VCG, and the absence of NeuroD1 results in the failure of these neurons to differentiate properly and survive after generated [7,10]. NeuroD1 also functions downstream of MATH-1 in the development of the cerebellum. MATH-1 is absolutely required for the generation and maintenance of the dividing external granule layer (EGL) [2] of the cerebellum. Moreover, after they are formed, the EGL cells require NeuroD1 for their survival at later stages [8]. Therefore, it seems that the epistatic partners of bHLHs may differ depending on the subtype of neurons.

Abbreviations: bHLH, basic helix-loop-helix; CNS, central neural system; EGL, external granule layer.

* Address: College of Pharmacy, Sookmyung Women's University, Seoul 140-742, Republic of Korea. Fax: +82 2 2077 7614.
E-mail address: wykim@sookmyung.ac.kr

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2012.01.100

Although it is clear that Neurogenins and Math1 function upstream of NeuroD1, the downstream targets of NeuroD1 have yet to be clearly defined. In this regard, NSCL1(Nhlh1) and NSCL2(Nhlh2), the neuronal homologs of TAL1/SCL1, another bHLH transcription factor that is required for vasculogenesis and primitive hematopoiesis [11,12], are interesting. Similar to TAL/SCL, which functions at a very early stage of hemangiogenesis [13,14], the role of NSCL in neurogenesis was originally suggested to be a very early determining factor, as is Neurogenin [15]. However, the ensuing research has suggested a redundant role or different epistatic relationships for these two transcription factors in mice. For example, NSCL1 and NSCL2 are co-expressed with NeuroD1 in the inner layer of the EGL [16–18]. Although NeuroD1 expression persists in fully differentiated granule cells, the expression of both NSCL1 and NSCL2 disappears soon after the birth [17,18]. In addition, knock-out mouse experiments for NSCL1, NSCL2, and NeuroD1 suggested that NSCL1 and NSCL2 are not early determining bHLHs but are later, postmitotic bHLHs [19–22]. However, no conclusive results regarding the functional and expressional relationship between these two NSCL and NeuroD families have been reported.

In the study presented herein, NeuroD1 is shown to be an upstream regulator of NSCL1 expression, but not NSCL2 expression, using both gain-of-function and loss-of-function studies that utilized an in vitro cell culture system and an in vivo single/double knock-out mouse system, respectively.

2. Materials and methods

2.1. In situ hybridization of ND1 KO mouse and ND1/ND2 compound KO mouse cerebella

All of the animal procedures were conducted with the approval of the University of Colorado Institutional Animal Care and Use Committee. NeuroD1 KO mice ($ND^{-/-}$) [8] and NeuroD2 KO mice ($ND2^{-/-}$) [23] have been previously reported. Both strains were maintained in the 129/SV background. Single heterozygotes of both strains ($ND^{+/-}$ and $ND2^{+/-}$) were mated to generate double heterozygotes ($ND^{+/-}$ and $ND2^{+/-}$), and these double heterozygous mice were crossed to generate the double KO mice ($ND^{-/-}$; $ND2^{-/-}$) and other genotypes. The detailed phenotype of the double KO mice will be reported elsewhere. The cerebella of the pups were dissected on the day they were born (P0), fixed in 4% paraformaldehyde for 6 h and then immersed in sucrose O/N. The 14 μm cryo-sections were prepared and used for in situ hybridization assays, as reported previously [7]. The ND and genotype were confirmed by Southern blotting [8] and PCR using the following primers, 5′-accatgcactctgtacgcatt-3′, 5′-gaaaactgagacactcatctg-3′, and 5′-aaacgccgagttaaagccatc-3′. ND2 genotypes were done by PCR as described before [23].

2.2. RT-PCR and Northern blotting

Total RNA was purified from the newborn mice cerebella or transfected cell lines with the Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The RT-PCR for NSCL1 was performed as previously reported [19]. The NSCL2 RT-PCR primers were 5′-cattctcaaccatgtcctggacg-3′ and 5′-ggggttaaagtctcaccaagctc-3′. A 1 μg aliquot of total RNA was reverse transcribed with SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA) using poly T primers in a 20 μl reaction. One microliter of the reaction products was used in the subsequent PCR. Actin RT-PCR was done as described previously [24]. Northern blotting was performed using standard methods with a formaldehyde-denatured gel, and radioisotope-labeled probes.

2.3. Cell culture, vector construction and transfection

Neuro2A mouse neuroblastoma cells were cultured in DMEM in the presence of 10% of fetal bovine serum. Plasmids were transfected into Neuro2A cells and after 2 days, the RNA was purified. The AQ mutant of the NeuroD1 protein was generated by the substitution of $E^{110}R^{111}$ in the basic region with $A^{110}Q^{111}$ using PCR and the sequences were verified by sequencing. The AQ substitution has previously been shown to disrupt the DNA-binding activity of many closely related bHLH proteins, including NeuroD2 [25] and Neurogenin1 [26]. In the Western blot, the band for the mutant protein was indistinguishable from that for wild-type NeuroD1.

3. Results

3.1. NeuroD1 and NeuroD2 activate NSCL1 transcription in a murine neuroblastoma cell line

To determine whether NeuroD1 can activate NSCL1 transcription in mouse neuronal cells, NeuroD1 was ectopically expressed in the Neuro2A murine neuroblastoma cell line, and the expression of the endogenous NSCL1 mRNA was analyzed by Northern blot analysis. As shown in Fig. 1A, NeuroD1 activated the expression of endogenous NSCL1. NeuroD2, a close homolog of NeuroD1, also increased the expression of NSCL1, whereas a myogenic bHLH factor, myoD, did not. To determine whether NSCL1 gene activation depends on the transcriptional activity of NeuroD1 and not on the sequestration of the inhibitor bHLH protein, two mutations ($NeuroD1^{AQ}$) were introduced into the basic region [25]. Because these two amino acids were essential for the DNA binding of many

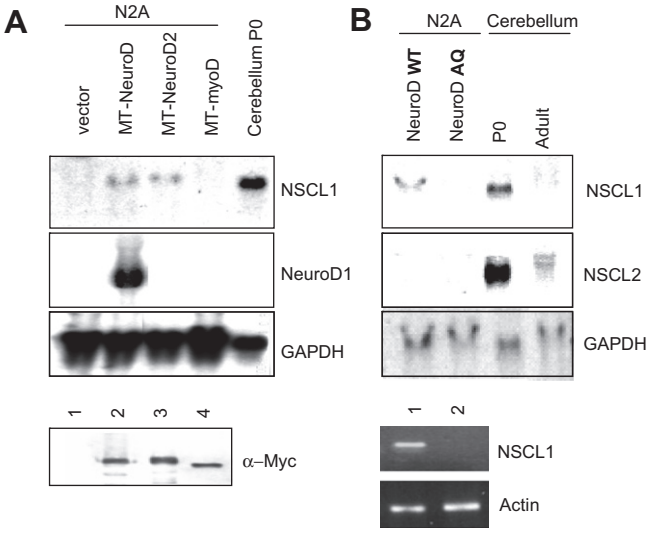

Fig. 1. In vitro activation of NSCL by NeuroD. (A) NeuroD1 and NeuroD2 can induce the endogenous expression of NSCL1 in the Neuro2A cell line. PCS2MT (Myc-tag) empty vector, lane 1; PCS2MT-NeuroD1, lane 2; PCS2MT-NeuroD2, lane 3; PCS2MT-MyoD, lane 4. The closed and opened arrowheads represent the sizes of the 28S and 18S rRNAs, respectively. (B) The expression of NSCL1 requires the DNA-binding affinity of NeuroD1, and NeuroD1 does not activate NSCL2. Expression constructs for the wild-type protein (WT) and the DNA-binding mutant (AQ) were transfected into the Neuro2A cell line, and the RNA was purified and used for Northern blotting (top) and RT-PCR (bottom). The wild type (but not the mutant) induced the expression of NSCL1. NSCL2 was not activated by any of the transfected plasmids. The NSCL1 mRNA was not detected in the mutant transfected. The RNA pools isolated from newborn mouse cerebella (P0) and adult cerebella were used as positive and negative control, respectively, for the NSCL Northern blot.

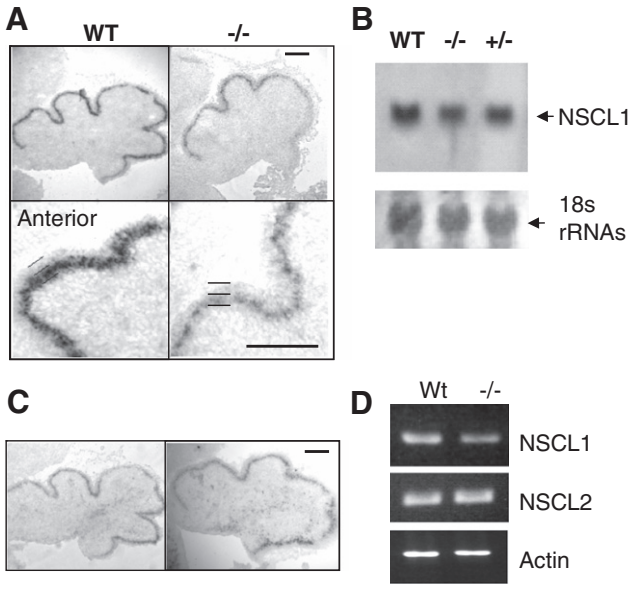

Fig. 2. NSCL1 expression decreased in the absence of NeuroD1, but NSCL2 expression did not. (A) In situ hybridization of NSCL1 in NeuroD1-null and wild-type P0 cerebella. The scale bars represent 100 μM. (B) Northern blotting of NSCL1 from NeuroD1-null and wild-type P0 cerebella. (C) In situ hybridization of NSCL2 in NeuroD1-null and wild-type P0 cerebella. (D) RT-PCR analysis of NSCL1 and NSCL2 in the NeuroD1-null P0 cerebellum.

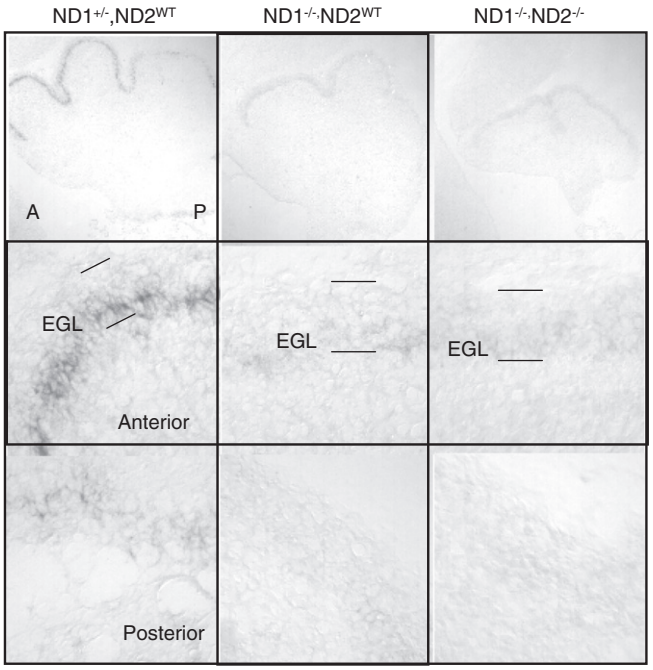

Fig. 3. The expression of NSCL1 in the cerebella of newborn mice requires NeuroD1 but not NeuroD2. The NeuroD1 and NeuroD2 compound knock-out mouse cerebella were used for the in situ hybridization of NSCL1. Only the NeuroD1 mutation affected the expression of NSCL1 in the extra granule cell layer (EGL) of the cerebellum, in which the expression is stronger in the anterior (A) than in the posterior (P) region. The NeuroD2 mutation did not eliminate the low residual expression of NSCL1 in the NeuroD1 mutant cerebella.

vertebrate bHLH transcription factors, including NeuroD2 and Neurogenin [25], this mutation should completely disrupt the DNA-binding ability of NeuroD1 [27]. The expression vector for the mutant NeuroD1^{AQ} was transfected into the Neuro2A cells, which were then examined for the expression of NSCL1 by

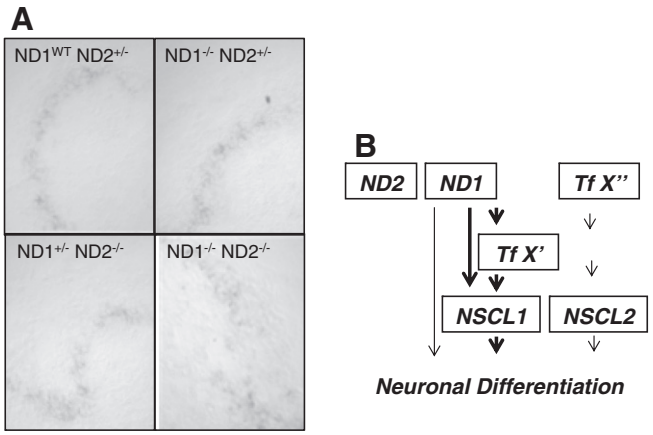

Fig. 4. Regulation of NSCL2 expression by NeuroD2 and the model of NSCL1/2 regulation. (A) NSCL2 expression does not require either NeuroD1 or NeuroD2 in vivo. The cerebella from P0 compound KO mice were examined by in situ hybridization for NSCL2. The expression of NSCL2 was not changed by the mutation of NeuroD1 or NeuroD2. Even in the compound-null mice, a significant amount of expression was detected. (B) The model for the regulatory pathway involving the NeuroD family and the NSCL family. Tf X′ amd Tf X″ indicate unknown factors. The bold arrows indicate the pathway supported by this study.

Northern analysis and RT-PCR. Fig. 4B shows that NeuroD1^{AQ} failed to activate the transcription of endogenous NSCL1 in NEURO2A cells. As previously reported, the newborn mice cerebella expressed NSCl1 and NSCL2, whereas the expression of NSCL1 was undetectable and that of NSCl2 was weak in adult mice cerebella. A similar experiment was performed using a truncated form of NeuroD1 in which the C-terminal region of the DNA-binding domain was truncated. This mutant was also unable to induce NSCL1 expression (data not shown). These results indicate that the DNA-binding domain and the transcriptional activity of NeuroD1 are required for the induction of NSCL1 transcription in Neuro2A cells. Interestingly, the transcription of NSCL2 was not activated by either NeuroD1 or NeuroD2, as assessed by Northern analysis and RT-PCR.

3.2. NeuroD1 is required for the proper expression of NSCL1 during mouse cerebellum development but not for the expression of NSCL2

We next examined the transcriptional relationship between NSCL and NeuroD1 in developing mouse cerebella. Because it has been previously reported that NeuroD1 expression is unchanged in NSCL1-null cerebella [19], in situ hybridization was performed on NeuroD1-null [8] neonatal pups to determine whether the NSCL gene expression is altered in the NeuroD1-null mouse cerebella. In the wild-type EGL, the NSCL1 expression is always stronger in the anterior lobules than in the posterior lobules (Fig. 2), and the NSCL1 expression was significantly decreased in the NeuroD1-null EGL relative to the control mouse EGL. In addition, as is the case with the wild-type EGL, the remaining NSCL1 expression was relatively higher in the anterior region of the EGL. The number of cells expressing NSCL1 in the wild-type and mutant mice did not change at this stage, but the intensity of the signal reflects the level of expression.

To confirm the decrease in the NSCL1 expression in the NeuroD null cerebella, Northern blot analysis was performed on P0 cerebella. A band for NSCL1 of approximately 4 kb produced a weak signal in the NeuroD1$^{+/-}$ cerebella, and the band intensity was further decreased for the NeuroD1$^{-/-}$ cerebella, confirming the reduction of NSCL1 mRNA due to the absence of NeuroD1. NSCL2, which has an expression pattern similar to that of NSCL1 in the cerebellum according to many previous studies, was then evaluated to determine if it is regulated by NeuroD1 (Fig. 2C). In the wild-type

cerebella, the expression of NSCL2 is slightly stronger in the posterior region of the cerebellum, which a pattern that is opposite that of NSCL1. Neither the expression level nor the pattern of NSCL2 changed significantly in the NeuroD1$^{-/-}$ cerebellum, as assessed by in situ hybridization. Last, that the regulatory pathways of NSCL1 and NSCL2 differ due to the absence of NeuroD1 was confirmed by performing RT-PCR for NSCL1 and NSCL2 mRNAs (Fig. 2D). Again, reductions in the NSCL1 mRNA level in the NeuroD-null cerebella sample were observed.

3.3. NeuroD2 does not compensate for the role of NeuroD in NSCL1 expression and is not required for NSCL2 expression

NeuroD2, which is a close relative of NeuroD1 with respect to both the protein sequence and the expression pattern, also plays an important role during cerebellum development. In the absence of NeuroD2, the cerebellum size is reduced, and the expression of selective genes, including BDNF, is reduced [23]. Because NeuroD2 also activated NSCL1 in the Neuro2A transfection assay (Fig. 1A), it was postulated that NeuroD2 may be responsible for the remaining expression of NSCL1 in the NeuroD$^{-/-}$ cerebella. To confirm this hypothesis, NeuroD1 and NeuroD2 double-null progeny were generated, and the level of NSCL1 expression was examined by in situ hybridization (Fig. 3). The expression level was lower in ND1$^{-/-}$; ND2^{wt} mice than in ND1$^{+/-}$; ND2^{wt} mice; however, there was no further decrease in ND1$^{-/-}$; ND2$^{-/-}$ mice. In the anterior lobule, the EGL cells express more NSCL1 than the posterior lobule EGL cells in all of the genotypes examined. These results suggest that the level and pattern of NSCL1 expression was not affected by the absence of NeuroD2.

We again examined the possibility that NeuroD2 regulates NSCL2 expression, as NeuroD1 regulates NSCL1 expression in vivo; the regulation of NSCL2 expression by NeuroD2 would explain why the NSCL2 expression did not change in the NeuroD1-null cerebella. However, the in situ hybridization results showed no clear correlation between the NeuroD2 mutation and NSCL2 expression, suggesting that NSCL2 expression is not regulated by NeuroD1 or NeuroD2.

Collectively, the in situ hybridization analysis of NeuroD1 and NeuroD2 null cerebella and the results from the compound null mice demonstrated that NeuroD1 regulates NSCL1 expression, but not NSCL2 expression, independently of NeuroD2.

4. Discussion

NeuroD1 is widely expressed in many CNS neurons during development. Although several bHLH proteins (e.g., Neurogenins and Math1) are believed to act upstream of NeuroD1 during neuronal development [2,3,5,7,9], the downstream targets of NeuroD1 are not well understood. The potential downstream target genes of NeuroD1 include other bHLH transcription factors; however, none of these have been identified. In the study presented herein, we addressed whether two neuronal bHLH transcription factors, NSCL1 and NSCL2, which are closely linked with NeuroD1 in the developing nervous system, are under the regulation of NeuroD.

The epistatic relationship between these two NeuroDs and NSCL1 was suggested a decade ago [15]: Bao et al. suggested that NSCL1 is an upstream regulator of NeuroD1 in Xenopus. However, the report that NeuroD1 expression was unchanged in NSCL1/NSCL2 mice suggested that NSCLs may not act upstream of NeuroD1 [19]. In contrast, the results in the current study showed that the NSCL1 expression decreased in the absence of NeuroD1 at the transcriptional level. Interestingly, the expression of *NSCL2* remained unchanged. The present and previously reports [19] support the hypothesis that NeuroD1 acts upstream of NSCL1 expression.

NeuroD2, a close homolog of NeuroD1, induced the transcription of NSCL1 in Neuro2A cells, suggesting that NeuroD2 may regulate NSCL1 gene expression (Fig. 1). In that case, the residual amount of NSCL1 expression found in the NeuroD1-null cerebella could be due to the presence of NeuroD2 in the NeuroD1-null EGL. However, the unchanged expression of NSCL1 in the NeuroD1/NeuroD2 double-null cerebella, relative to the expression in NeuroD1 single-null cerebella indicates that NeuroD2 may play a minimal role, if any, in the regulation of NSCL1 expression in the cerebellum in vivo. Another interpretation of this result could be that perhaps NeuroD1 regulates the expression of NeuroD2 in the cerebellum and that the NeuroD1-null cerebella failed to express NeuroD2, similar to trophoblasts [28]. However, this may not be the case because NeuroD1 and NeuroD2 are compensating or independent of each other rather than in sequential regulation during neuronal development. (Kim unpublished data [7,8,23]). The expression of the *NSCL1* gene may also be regulated by other transcription factors that are not related to NeuroD. In addition, *NSCL1* expression might be directly regulated by different factors in the different subsets of neurons other than the cerebellar EGL. The possible sequential regulation based on the current study is summarized in Fig. 4B.

The data reported herein support the hypothesis that NeuroD1 acts upstream of NSCL1, potentially as a direct transcriptional regulator in mouse cerebella; however, the regulation of these two factors can be different in other neuronal tissues. It is also possible that there are other factors regulating NSCL1. MATH3 and MATH2 are factors that are closely related to NeuroD1 and NeuroD2, yet they are not related to NSCL expression because they are not normally expressed in the postnatal EGL. Nevertheless, it is still possible that these factors were aberrantly activated in the absence of NeuroD1. Further investigation is warranted.

The report of Bao was interesting because an LMO protein (XLMO3) and a bHLH protein (Xnscl) were co-expressed, and synergistically functioned during vertebrate neurogenesis, as LMO-2 and SCL1 do in the hematopoietic system [29,30]. These authors suggested that XNSCL/LMO is key factor in neuronal determination and is the most upstream factor in this pathway, upstream of even Neurogenin. However, the expression pattern and loss of function studies suggest that the function of NSCL1 during neurogenesis will be different from the pivotal role of SCL1 in hematopoietic stem cells, at least in mice. The neurogenin family is expressed at the very first stage of neuronal cell fate determination in many cranial and dorsal root ganglia that express NeuroD1 at a later stage. In mammalian systems, no evidence is yet available to elucidate the function of NSCLs in the very early stage of neurogenesis. The finding that NeuroD1 regulates NSCL1 expression suggests that NSCL1 may act at the terminal stages of mammalian neurogenesis and not at the early, fate determination stages.

Math1 is upstream of NeuroD1 during granule cell development in the cerebellum. Interestingly, Math1 and NeuroD1 are expressed at the same time in ear hair cells during development, but the requirement for bHLHs during hair cell development is quite different—Math1 is essential for hair cell development, but NeuroD1 is not [3,7]. NeuroD1, the downstream target of Math1, may have an independent role that is unrelated to the survival or differentiation of the inner ear hair cells, as NSCL1/2 double-null hair cells showed normal development, as did the NeuroD1-null hair cells. Neurogenin is upstream of NeuroD1 in the development of vestibulocochlear ganglia (VCG, VII–VIII). The role of NSCL1 and NSCL2 in the developing VCG ganglion was recently reported using double-null mice, and the expression pattern and phenotype suggested the functional redundancy of these transcription factors with NeuroD1 [7,21]. However, the phenotype of the NSCL1/2 double-null mice during VCG development was much milder than that for NeuroD1, and the cerebellar defects of the NSCL1/2 double-null mice were much milder than those of NeuroD1-null mice [8,22].

In this study, the epistatic regulation of NSCL1 expression by that NeuroD1 was demonstrated, and it was found that NeuroD2 and NSCL2 are not directly involved in this regulation. Further study is warranted regarding the involvement of other transcription factors and the correlation in other tissue subtypes. The investigation of the promoter of NSCL1 and the identification of promoter elements that are bound and activated by NeuroD1 are also needed.

Acknowledgments

I thank Dr. Jacquelin Lee for her unwavering support and encouragement, which enabled the publication of this study. I also thank Dr. Marcora for the construction of the NeuroD1^{AQ} plasmid and Dr. Woo for technical assistance. I am indebted to Dr. Tapscott for the NeuroD2-null mice. This work was supported in part by the Sookmyung Women's University Research Grants 2011.

References

[1] H. Weintraub, The MyoD family and myogenesis: redundancy, networks, and thresholds, Cell 75 (1993) 1241–1244.
[2] N. Ben-Arie, H.J. Bellen, D.L. Armstrong, A.E. McCall, P.R. Gordadze, Q. Guo, M.M. Matzuk, H.Y. Zoghbi, Math1 is essential for genesis of cerebellar granule neurons, Nature 390 (1997) 169–172.
[3] N.A. Bermingham, B.A. Hassan, S.D. Price, M.A. Vollrath, N. Ben-Arie, R.A. Eatock, H.J. Bellen, A. Lysakowski, H.Y. Zoghbi, Math1: an essential gene for the generation of inner ear hair cells, Science 284 (1999) 1837–1841.
[4] C. Fode, G. Gradwohl, X. Morin, A. Dierich, M. LeMeur, C. Goridis, F. Guillemot, The bHLH protein NEUROGENIN 2 is a determination factor for epibranchial placode-derived sensory neurons, Neuron 20 (1998) 483–494.
[5] Q. Ma, C. Kintner, D.J. Anderson, Identification of neurogenin, a vertebrate neuronal determination gene, Cell 87 (1996) 43–52.
[6] B. Fritzsch, D.F. Eberl, K.W. Beisel, The role of bHLH genes in ear development and evolution: revisiting a 10-year-old hypothesis, Cell Mol. Life Sci. 67 (2010) 3089–3099.
[7] W.Y. Kim, B. Fritzsch, A. Serls, L.A. Bakel, E.J. Huang, L.F. Reichardt, D.S. Barth, J.E. Lee, NeuroD-null mice are deaf due to a severe loss of the inner ear sensory neurons during development, Development 128 (2001) 417–426.
[8] T. Miyata, T. Maeda, J.E. Lee, NeuroD is required for differentiation of the granule cells in the cerebellum and hippocampus, Genes Dev. 13 (1999) 1647–1652.
[9] Q. Ma, Z. Chen, I. del Barco Barrantes, J.L. de la Pompa, D.J. Anderson, Neurogenin1 is essential for the determination of neuronal precursors for proximal cranial sensory ganglia, Neuron 20 (1998) 469–482.
[10] M. Liu, F.A. Pereira, S.D. Price, M.J. Chu, C. Shope, D. Himes, R.A. Eatock, W.E. Brownell, A. Lysakowski, M.J. Tsai, Essential role of BETA2/NeuroD1 in development of the vestibular and auditory systems, Genes Dev. 14 (2000) 2839–2854.
[11] C.G. Begley, S. Lipkowitz, V. Gobel, K.A. Mahon, V. Bertness, A.R. Green, N.M. Gough, I.R. Kirsch, Molecular characterization of NSCL, a gene encoding a helix-loop-helix protein expressed in the developing nervous system, Proc. Natl. Acad. Sci. USA 89 (1992) 38–42.
[12] V. Gobel, S. Lipkowitz, C.A. Kozak, I.R. Kirsch, NSCL-2: a basic domain helix-loop-helix gene expressed in early neurogenesis, Cell Growth Differ. 3 (1992) 143–148.
[13] R.A. Shivdasani, E.L. Mayer, S.H. Orkin, Absence of blood formation in mice lacking the T-cell leukaemia oncoprotein tal-1/SCL, Nature 373 (1995) 432–434.
[14] J.E. Visvader, Y. Fujiwara, S.H. Orkin, Unsuspected role for the T-cell leukemia protein SCL/tal-1 in vascular development, Genes Dev. 12 (1998) 473–479.
[15] J. Bao, D.A. Talmage, L.W. Role, J. Gautier, Regulation of neurogenesis by interactions between HEN1 and neuronal LMO proteins, Development 127 (2000) 425–435.
[16] M.K. Duncan, L. Bordas, E. Dicicco-Bloom, K.K. Chada, Expression of the helix-loop-helix genes Id-1 and NSCL-1 during cerebellar development, Dev. Dyn. 208 (1997) 107–114.
[17] M.F. Haire, A. Chiaramello, Transient expression of the basic helix-loop-helix protein NSCL-2 in the mouse cerebellum during postnatal development, Brain Res. Mol. Brain Res. 36 (1996) 174–178.
[18] M. Uittenbogaard, D.R. Peavy, A. Chiaramello, Expression of the bHLH gene NSCL-1 suggests a role in regulating cerebellar granule cell growth and differentiation, J. Neurosci. Res. 57 (1999) 770–781.
[19] M. Kruger, T. Braun, The neuronal basic helix-loop-helix transcription factor NSCL-1 is dispensable for normal neuronal development, Mol. Cell. Biol. 22 (2002) 792–800.
[20] M. Kruger, K. Ruschke, T. Braun, NSCL-1 and NSCL-2 synergistically determine the fate of GnRH-1 neurons and control necdin gene expression, EMBO J. 23 (2004) 4353–4364.
[21] M. Kruger, T. Schmid, S. Kruger, E. Bober, T. Braun, Functional redundancy of NSCL-1 and NeuroD during development of the petrosal and vestibulocochlear ganglia, Eur. J. Neurosci. 24 (2006) 1581–1590.
[22] T. Schmid, M. Kruger, T. Braun, NSCL-1 and -2 control the formation of precerebellar nuclei by orchestrating the migration of neuronal precursor cells, J. Neurochem. 102 (2007) 2061–2072.
[23] J.M. Olson, A. Asakura, L. Snider, R. Hawkes, A. Strand, J. Stoeck, A. Hallahan, J. Pritchard, S.J. Tapscott, NeuroD2 is necessary for development and survival of central nervous system neurons, Dev. Biol. 234 (2001) 174–187.
[24] S. Alonso, A. Minty, Y. Bourlet, M. Buckingham, Comparison of three actin-coding sequences in the mouse; evolutionary relationships between the actin genes of warm-blooded vertebrates, J. Mol. Evol. 23 (1986) 11–22.
[25] M.H. Farah, J.M. Olson, H.B. Sucic, R.I. Hume, S.J. Tapscott, D.L. Turner, Generation of neurons by transient expression of neural bHLH proteins in mammalian cells, Development 127 (2000) 693–702.
[26] Y. Sun, M. Nadal-Vicens, S. Misono, M.Z. Lin, A. Zubiaga, X. Hua, G. Fan, M.E. Greenberg, Neurogenin promotes neurogenesis and inhibits glial differentiation by independent mechanisms, Cell 104 (2001) 365–376.
[27] C.H. Park, J.S. Kang, J.S. Kim, S. Chung, J.Y. Koh, E.H. Yoon, A.Y. Jo, M.Y. Chang, H.C. Koh, S. Hwang, H. Suh-Kim, Y.S. Lee, K.S. Kim, S.H. Lee, Differential actions of the proneural genes encoding Mash1 and neurogenins in Nurr1-induced dopamine neuron differentiation, J. Cell Sci. 119 (2006) 2310–2320.
[28] B.A. Westerman, A. Chhatta, A. Poutsma, T. van Vegchel, C.B. Oudejans, NEUROD1 acts in vitro as an upstream regulator of NEUROD2 in trophoblast cells, Biochim. Biophys. Acta 1676 (2004) 96–103.
[29] H. Osada, G. Grutz, H. Axelson, A. Forster, T.H. Rabbitts, Association of erythroid transcription factors: complexes involving the LIM protein RBTN2 and the zinc-finger protein GATA1, Proc. Natl. Acad. Sci. USA 92 (1995) 9585–9589.
[30] I.A. Wadman, H. Osada, G.G. Grutz, A.D. Agulnick, H. Westphal, A. Forster, T.H. Rabbitts, The LIM-only protein Lmo2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins, EMBO J. 16 (1997) 3145–3157.