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The Role of *BETA2/NeuroD1* in the Development of the Nervous System

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Abstract

BETA2/NeuroD1 is a member of the basic helix-loop-helix (bHLH) transcription factor family, which has been shown to play a major role in development of the nervous system and formation of the endocrine system. Gain-of-function studies have indicated that *BETA2/NeuroD1* is important for the neurogenesis of *Xenopus* embryos and several neurogenic cell lines. Disruption of the gene encoding *BETA2/NeuroD1* leads to severe abnormalities of the developing mouse central nervous system as well as the peripheral nervous system. The focus of this article is on the recent progress in understanding the role of *BETA2/NeuroD1* in the development of the nervous system.

Index Entries: *BETA2*; *NeuroD1*; bHLH; CNS; PNS; neurogenesis.

Introduction

During nervous system development, multipotent progenitor cells give rise to various kinds of neuronal cell types, depending on their context. The development of diverse types of neuronal cells can be regulated positively or negatively by multiple basic helix-loop-helix (bHLH) transcription factors in a cascade manner. The tissue-specific neuronal bHLH factor family regulates multiple aspects of neurogenesis. Most of the proteins in this family, especially class B bHLH factors whose expression is cell-type restricted, have both DNA binding and helix-loop-helix (HLH) protein dimerization domains. These proteins usually heterodimerize with ubiquitously expressed bHLH factors, such as E12/E47, via the HLH domain, and bind the specific E-box DNA sequence (CANNTG) of target genes, via their basic DNA binding domains. In this way, bHLH transcription

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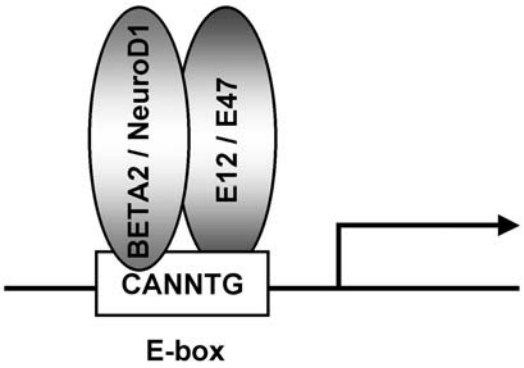

- Endocrine-related genes
  - Insulin (*7*)
  - Proopiomelanocortin (*9*)
  - Somatostatin transactivating factor 1 (*10*)
  - Secretin (*11*)
  - Sulfonylurea receptor I (*12*)
- Neuronal differentiation-related genes
  - Adenylate kinase isozyme 1 gene (*13*)
  - Inositol 1,4,5-Triphosphate receptor 1 (*14*)
  - Early B-cell factor 3 (*15*)
  - Brn3d (*16*)

Fig. 1. Target genes of *BETA2/NeuroD1*.

factors control cell fate determination and differentiation from invertebrates to mammals *(1,2).* Vertebrate proneural bHLH genes can be divided into two major groups, *Ash* and *Ath*, based on their function and homology to the *Drosophila* proneural genes. The *Ash* group has a well-conserved bHLH amino acid sequence with homology to the *Drosophila Acheate-Scute Complex (AS-C)* gene *(3).* On the other hand, proteins in the *Ath* group are highly homologous to the *Drosophila atonal* proneural gene *(4). BETA2/NeuroD1* is considered to be a member of the *Ath* group, which also includes the *Math* and *Neurogenin* subfamilies *(5–8).*

Taking advantage of its ability to heterodimerize with E12/E47, two independent laboratories cloned *BETA2/NeuroD1* by using the yeast-two-hybrid system to clone BETA2/NeuroD1 *(6,7). BETA2*, β-cell E-box transactivator 2, was first isolated in our group as a β-cell specific transactivator *(7).* Northern blot analysis demonstrated that a 2.6-kb message corresponding to *BETA2* can be detected in pancreatic endocrine cells, several endocrine cell lineages in the intestine as well as in the brain, suggesting that *BETA2* may act as an important regulator of endocrine and neuronal cells *(7).* This gene was also isolated as a neurogenic differentiation factor *(NeuroD)* from frog and mouse embryos. Ectopic expression of *NeuroD* in *Xenopus* embryos can convert both non-neuronal populations of neural crest cells and presumptive epidermal cells into neurons *(6).*

The function of *BETA2/NeuroD1* has been studied as an important transcription regulator of genes in endocrine cells, enteroendocrine cells, and neuroendocrine cells. These target genes include insulin *(7),* proopiomelanocortin *(POMC) (9),* somatostatin transactivating factor-1 *(STF-1)*/pancreatic duodenal homeobox gene-1 *(PDX-1) (10),* secretin *(11),* and sulfonylurea receptor 1 *(SUR1) (12).* In addition, *BETA2/NeuroD1* is required for upregulation of neuronal differentiation-related genes, such as adenylate kinase isozyme 1 *(AK1) (13),* inositol 1,4,5-triphosphate receptor 1 *(IP3R1) (14),* early B-cell factor 3 *(Ebf3) (15),* and a POU domain transcription factor *(Brn3d) (16)* (Fig. 1). Therefore, *BETA2/NeuroD1* was likely to play an important role not only in development of neuroendocrine cells but also in the differentiation of neuronal cells.

In order to determine whether *BETA2/NeuroD1* was necessary for the normal development of the endocrine and nervous systems, a *BETA2* knockout mouse was generated by homologous recombination to replace the *BETA2* coding region with a β-galatosidase reporter gene *(17).* Mice lacking *BETA2/NeuroD1* die within 5 d postnatally due to severe diabetes mellitus with high ketone levels in the urine as a result of the loss of insulin-producing pancreatic β-cells *(17). BETA2/NeuroD1* knockout mice also failed to

develop the secretin- and cholecytokinin-producing enteroendocrine cells *(17,18).* When *BETA2/NeuroD1* knockout mice were first generated, it was difficult to study the role of the *BETA2/NeuroD1* gene in postnatal nervous system development because of perinatal lethality. However, surviving *BETA2/NeuroD1* knockout mice were obtained in a different genetic background *(19). BETA2/NeuroD1* null mice were also rescued by ectopic expression of insulin in the pancreas *(20).* The surviving *BETA2/NeuroD1* mutant mice allowed us to investigate the importance of the *BETA2/NeuroD1* gene in the nervous system through adulthood. The *BETA2/NeuroD1* gene was demonstrated to be necessary for the normal development of the granule cells in the dentate gyrus and cerebellum *(19,20)* as well as sensory neuronal cells and ganglion development of the inner ear *(21,22)* and the maintenance of photoreceptor cells in the retina *(23).* In this review, we will focus on recent progress in elucidating the roles of *BETA2/NeuroD1* in the development of the nervous system.

BETA2/NeuroD1 as a Differentiation Factor

Many bHLH transcription factors have been investigated in *Drosophila* and mammalian neurogenesis. These factors are well conserved structurally and functionally from *Drosophila* to mammals *(2).* However, some of the *Drosophila* bHLH genes, such as *achaete, hairy,* and *E(spl),* play important roles in cell type determination in *Drosophila* neurogenesis but its counterparts play only a role as a differentiation factor in mammalian neurogenesis *(24,25).* During neurogenesis, bHLH factors are sequentially expressed in certain cells and either positively or negatively regulate cell type determination and differentiation *(26,27).* Balanced expression between positive and negative regulators and their stepwise expression are very important for the morphogenesis of the nervous system. Missed expression or overexpression of certain genes may cause severe morphological defects in various tissues, especially in the nervous system *(26,28–30).*

Neurogenin and *Mash1* are expressed early in undifferentiated, proliferating neural precursor cells *(31,32)* and can be considered as vertebrate neuronal determination genes. By contrast, *NeuroM,* a neuronal-specific *Ath* group member, is expressed in cells that have recently exited the mitotic cycle *(33)* and can be considered a neuronal differentiation factor. For the most part, *BETA2/NeuroD1* is detected in the developing nervous system where differentiating postmitotic neurons are distributed *(6).* However, ectopically expressed *BETA2/NeuroD1* can convert both non-neuronal populations of neural crest cells and presumptive epidermal cells into neurons *(6,34).* Furthermore, *BETA2/NeuroD1* expression has been detected in a few proliferating neuronal cells during nervous system development *(19,21, 35).* Therefore, *BETA2/NeuroD1* may contribute to multiple levels of neural development; nevertheless, it is believed that *BETA2/NeuroD1* mainly plays important roles in the terminal differentiation of postmitotic neuronal cells.

The suggested role of *BETA2/NeuroD1* in differentiation is supported by in vitro experiments. Retinoic acid (RA)-treated P19 embryonal carcinoma cells transiently expressed high levels of *Mash1, Math1, BETA2/NeuroD1,* and *NSCL-2* during neural cell differentiation *(36).* Transient transfection of vectors expressing *Neurogenin1, NeuroD2,* and *Mash1* can convert mouse P19 embryonal carcinoma cells into differentiated neurons *(37).* Although the neurogenic potential of *BETA2/NeuroD1* was not as high in the P19 embryonal carcinoma cells when compared to the neurogenic potential of *Neurogenin1* and *Math1,* PC12 stable cell lines expressing *BETA2/NeuroD1* exhibited neurite outgrowth in nondifferentiating conditions *(13).* These results are consistent with our finding that transiently expressed full-length *BETA2/NeuroD1* in the F11 neuroblastoma cell line, a hybrid cell line between dorsal root ganglionic neurons and neuroblastoma N18TG2 *(38),* can induce neurite

outgrowth in nondifferentiation conditions. In addition, a dominant negative mutant of *BETA2/NeuroD1,* which has only the DNA-binding domain of *BETA2/NeuroD1,* can inhibit the neurite outgrowth induced by dibutyryl-cAMP (db-cAMP) in a dose-dependent manner *(39).* These results indicate that *BETA2/NeuroD1* plays an important differentiation role in cells committed to the neuronal fate.

BETA2/NeuroD1 Roles In Vivo

BETA2/NeuroD1 is detected in a subset of neuronal tissues such as the developing central nervous system *(6,19,20,35)* and auditory and vestibular system *(21,22)* (Table 1), and in the intestine and pancreas *(17). BETA2/NeuroD1* transcripts were first detected in the trigeminal ganglion in the developing brain at embryonic day 9 (E9), the otic vesicle of developing ear at E9 *(6,21),* and the neuroblastic layers of retina at E10.5 *(23).* High-level expression of *BETA2/NeuroD1* in the developing nervous system persists throughout postnatal development and remains at stable levels in the adult central and peripheral nervous system *(35).* The expression pattern of *BETA2/NeuroD1* in the early stages of the developing brain as well as in the mature adult brain suggests that *BETA2/NeuroD1* is important not only for the initiation of neuronal differentiation, but also for maintenance of the nervous system.

As we mentioned earlier, *BETA2/NeuroD1* knockout mice die shortly after birth due to severe diabetes and ketoacidosis *(17,40).* However, when we bred the heterzygote *BETA2/NeuroD1* mutants (+/–) to 129/SvJ mice, some of the homozygous *BETA2* mutants (–/–) can survive to adulthood *(19,21,40).* Surviving *BETA2/NeuroD1* mutants are somewhat hyperglycemic but do not have ketonuria, and their insulin levels return to normal levels around 4 wk after birth *(19,21,40).* Surviving *BETA2/NeuroD1* knockout mice show severe ataxia, hyperactivity, circling, and swaying head movement *(21).* Similar behavioral symptoms were observed in *BETA2/NeuroD1* knockout mice rescued with exogenous insulin expression *(20).* Histological analysis of *BETA2/NeuroD1* knockout mouse brains revealed that they have severe defects in granule cells of the cerebellum and dentate gyrus *(19,20)* and special sensory organs such as the inner ear *(21,22)* and eye *(23).* These defects are discussed in detail in the following sections.

Roles of *BETA2/NeuroD1* in Central Nervous System

Cerebrum

Cerebral Cortex

The cerebral cortex of mammals consists of diverse types of neuronal and glial cells, and expresses multiple bHLH factors during its development *(27,41). BETA2/NeuroD1* expression can be detected in primary culture of the cerebral cortex of E16 embryos. The expression level of *BETA2/NeuroD1* decreases with increasing days in the culture so that no *BETA1/NeuroD1* expression is detected after 18 d of culture *(42).* In the developing mouse central nervous system (CNS), transcripts of *BETA2/NeuroD1* are first detected around E8 in neural epithelium. At E10, high-level expression of *BETA2/NeuroD1* can be observed in the CNS, including forebrain, hindbrain, spinal cord, and dorsal root ganglia (DRG). In the forebrain, *BETA2/NeuroD1* expression is restricted to the postmitotic neurons in the subventricular zone but it is also strongly expressed in other regions, including the hindbrain, spinal cord, and DRG, at E12 *(6,33,35).* This specific subventricular postmitotic cell expression implies that *BETA2/NeuroD1* may play an important role in neuronal cell differentiation in the late stages of neurogenesis. Strong *BETA2/NeuroD1* expression in the cerebral cortex is sustained until E16 during the period when cellular migration from the mantle zone into the marginal zone occurs to form the cortical layer. By E18, the expression level of *BETA2/NeuroD1* has declined in the cerebral

Table 1
Summary of Expression Patterns and Defects of Various Nervous Systems of BETA2/NeuroD1 Knockout Mice

	Cerebral cortex	Hippocampus	Dentate gyrus	Cerebellum	Retina	Inner ear	Olfactory bulb	Offactory epithelium
Expression period	E10 to adult	E14 to adult	E14 to adult	E14 to adult	E10.5 to adult	E8.5 to adult	E10.5 to adult	E10.5 to adult
Expression profile in the developing embryo	postmitotic neuron in subventricular zone	pyramidal cells CA1to CA3	granule cells	cerebellar anlagen (E14), rhombic lip (E14.5), deep cerebellar nuclei (E14.5), ganular cells of pre-migrating zone of EGL (E16.5)	optic stalk (E10.5), outer half of neuroblastic layer, some of ganglionic cells in GCL (E16.5)	ventral part of otic vesicle (E9), vestibular sensory epithelium (E14), cochlear sensory epithelium (E15.5), VIII's ganglions	otic vesicle (E10.5), granule cells, and olfactory interneurons	olfactory sensory neurons
Expression profile in the adult	most of neurons in cerebral cortex	pyramidal cells in hippo-campus	granule cells of dentate gyrus	granule cells in EGL and IGL, deep cerebellar nuclei	rod- and cone-photoreceptors of ONL	not reported	granule cells in mitral cell layer	olfactory sensory neurons
Histological phenotype of *BETA2/NeuroD1* knockout mice	normal	normal	>95% of granule cell missing	overal cerebellar size reduced 75%, granule cell missing in posterior compartment (lobule VI to X), and less foliation	shortened outer and inner segment of retina, decreased synapse between ONL and INL, ONL missing after 18 months old	shortened cochlear duct, sensory epithelia malformation, degenration of VIII's ganglions	normal	normal
Increased apoptosis	no	no	yes	yes	yes	yes	no	yes
Neurological abnomality of *BETA2/NeuroD1* knockout mice	not reported	normal	spontaneous seizures	impaired motor coordination, abnormal righting reflex, ataxia	blindness	deafness, imbalance, circling, hyper-activity, abnormal righting reflex	not reported	not reported

cortex, but it persists in the adult cerebral cortex at a stable level *(35).*

There is considerable evidence that *BETA2/ NeuroD1,* like other proneural bHLH factors, governs the neural vs glial cell fate decision in a variety of neural tissues. Overexpression of bHLH factors such as *Xath5, Math5, Mash1,* and *BETA2/NeuroD1* in neural retina resulted in a reduction in glial cell types and an altered neuron vs glia ratio *(43–45).* However, in contrast to the results observed in neural retina study, retroviral-mediated overexpression of *BETA2/ NeuroD1,* and other positive regulatory bHLH genes such as *Mash1, Math2, Math3, Neurogenin1, Neurogenin2,* and *NeuroD2* did not result in defects in gliogenesis in the cerebral cortex *(46).* Furthermore, mice lacking *BETA2/ NeuroD1* appear to have normal morphology and no obvious cell type changing or cell death in the cerebral cortex (J.-H. Cho and M.-J. Tsai, unpublished data). Although much has been learned about the roles of *BETA2/ NeuroD1* in other types of neurons of several other nervous tissues, little information has been learned regarding its potential role in the cerebral cortex. It is likely that either other bHLH factors compensate for the loss of *BETA2/ NeuroD1* in the cortex of knockout mice or *BETA2/NeuroD1* does not play an essential role in cortex development.

Hippocampal Formation

BETA2/NeuroD1 is prominently expressed in the hippocampus, especially in the granule cells of the dentate gyrus and pyramidal cells in CA1 and CA3 after E14. This hippocampal expression of *BETA2/NeuroD1* increases gradually and finally reaches a very high level at the prenatal period *(35).* After birth, expression of *BETA2/NeuroD1* is more localized to immature dentate granule cells at the inner portion of the dentate gyrus and somewhat lower expression is observed in the mature granule cells *(47).* Restriction of *BETA2/NeuroD1* expression to immature cells suggests that it may regulate the downstream genes that are involved in granule cell maturation. *BETA2/NeuroD1* expression in 4-mo-old adults remains high in the hippocampus, but it is decreased dramatically in 12-mo-old mice *(48).*

Although *BETA2/NeuroD1* is known as a postmitotic differentiating factor during the late stage of neurogenesis, a couple of reports have indicated that almost one third of proliferating cells of the hippocampal dentate gyrus and the hilus, where granule cells are known to be highly proliferative *(49),* express *BETA2/NeuroD1 (35).* However, in our group, we found that only a few *LacZ*-positive (*BETA2*-expressing) cells were also bromodeoxyuridine (BrdU)-positive *(19).* The validity of the expression of *BETA2/ NeuroD1* in proliferating cells is not clear. Since the BrdU incorporation time extended over a prolonged period in these studies, it is possible that *BETA2/NeuroD1*-expressing cells containing BrdU are cells that have recently exited from the cell cycle following BrdU labeling and are starting to differentiate and express *BETA2/NeuroD1.* On the other hand, if it is indeed expressed in proliferating cells, it may act to initiate the exit from the cell cycle and start the cellular differentiation process.

As expected from the high expression level in the granule cell of the dentate gyrus, mice lacking the *BETA2/NeuroD1* gene have abnormal hippocampal formation *(19,20).* The hippocampus of *BETA2/NeuroD1*-mutant mice lacks granule cells in the dentate gyrus and has no organized hilus, but pyramidal cells in the hippocampus (Ammon's horn, CA1 to CA3), appear normal even though *BETA2/ NeuroD1* is expressed there *(19).* The lack of granule cells in the dentate gyrus is due to neither a defect in the early populations of Cajal-Retzius and radial glial cells that are required for proper morphogenesis of the dentate gyrus *(50)* nor impaired migration of precursor cells and newly born granule cells from the neuroepithelium to the dentate gyrus. Instead, it is due to a defect in the proper morphogenesis of the dentate gyrus. Consequently, there is an increased apoptosis in the granule cells and reduction of the proliferation of precursor cells *(19).* All these studies provide evidence that *BETA2/ NeuroD1* may play a key role in the maintenance of proliferating cells and the

maturation and maintenance of granule cells of the dentate gyrus.

In addition to *BETA2/NeuroD1, NEX/Math2* or *NDRF/NeuroD2* may also play a role in granule cell development. Close examination of *NEX/Math2* and *BETA2/NeuroD1* double-mutant mice display a more severe defect in the dentate gyrus than the *BETA2/NeuroD1* single mutant *(51),* although the dentate gyrus of the *NEX/Math2* mutant is apparently normal *(52).* In addition, *NDRF/NeuroD2* may also play an important role in maintaining granule cell differentiation since it is expressed in the dentate gyrus later then *BETA2/NeuroD1* and *NDRF/NeuroD2* mediated *prox-1* expression is affected in the *BETA2/NeuroD1* knockout mice *(19).* Thus, all these related genes may have some function in maintaining granule cells of the dentate gyrus, although *BETA2/NeuroD1* is most likely the major player in its differentiation.

Cerebellum

BETA2/NeuroD1 is expressed in the hindbrain throughout mouse embryogenesis. *BETA2/NeuroD1* transcripts are first detectable in the primordial cerebellum after E14 and reach high levels during the prenatal period *(35).* The noticeable increase in *BETA2/NeuroD1* expression in the cerebellum correlates with granule cell development *(53,54). BETA2/NeuroD1* is highly expressed in both external and internal granule cells of the cerebellum between P5 and P13. *BETA2/NeuroD1* expression persists to adulthood in the granule cell layer *(20,35).* During the active proliferation period of the granule cell, around P5, *BETA2/NeuroD1* is detected mainly in the postmitotic cells, although some was observed in the proliferating cells *(35).*

It has been reported that disruption of some of the bHLH genes, such as chick *neuronal stem cell leukemia 1 (cNSCL1),* lead to the abnormal morphology of the cerebral tectum and cerebellum *(55).* Loss of *BETA2/NeuroD1* has been shown to result in a severe reduction in the granule cell population of the cerebellum during postnatal development (M.-J. Tsai, unpublished data, *20*). In these mutant mice, overall size of the adult cerebellum decreases 75%. More specifically, granule cells in the posterior compartment of the cerebellum, lobule VII to X, are almost completely missing, while the population in the anterior compartment, lobule I to VI, is dramatically reduced. In addition, foliation is less apparent when compared to the wild-type littermates. The reduction in the size of the cerebellum in *BETA2/NeuroD1* knockout mice coincides with behavior problems such as impaired balance and abnormal righting reflex. However, the cerebellar abnormalities are restricted only to the granular cell population. Miyata et al. indicated that the decrease of granule cells is not concomitant with the loss of Purkinje cells or any other neuronal cells in the *BETA2/NeuroD1* knockout *(20).* In contrast, our unpublished observations indicate that there is a major decrease of Purkinje cells in the posterior lobes of *BETA2/NeuroD1* mutant mice.

Although the cerebellums of the wild-type and *BETA2/NeuroD1* knockout mouse are not much different in size and morphology at perinatal stages, abnormal foliation and size reduction become apparent after P2 (M.-J. Tsai, unpublished data; *20*). Apoptosis may be responsible for the overall size reduction of the cerebellum. Indeed, in *BETA2/NeuroD1* knockout mice, apoptotic cells have been observed in the premigrating zone of the inner half of the external granule cell layer of the posterior compartment of the cerebellum where postmitotic granule cells are confined at around P0 (M.-J. Tsai, unpublished data; *20*).

Roles of *BETA2/NeuroD1* in Sensory Nervous System

Retina

During embryogenesis, *BETA2/NeuroD1* is first expressed in scattered cells in the central portion of the developing retina at E10.5. The expression of *BETA2/NeuroD1* extends from the central to the lateral retina in both directions to the lateral ends. The expression peaks at

approximately E18.5 in outer half of the neuroblastic layer where differentiating photoreceptors are localized. By P3, most of the *BETA2/NeuroD1* expression was restricted to the outer nuclear layer (ONL) where photoreceptor cell bodies reside and slightly expressed in the inner nuclear layer (INL) where bipolar, horizontal, and amacrine cells are distributed. *BETA2/NeuroD1* was expressed predominantly in the ONL and remained at a stable level in the adult retina *(23). BETA2/NeuroD1* is expressed only in the differentiating or differentiated cells but not in proliferating cells in the retina *(23,56).* Birth-dating experiments showed that the role of *BETA2/NeuroD1* as a terminal differentiation factor; however, forced expression of *BETA2/NeuroD1* using viral vector in cultured retinal cells resulted in an increase of rod photoreceptors and amacrine cells, a decrease in bipolar interneurons, and a complete loss of Müller glial cells *(46),* indicating that *BETA2/NeuroD1* influences the neuron vs glial-cell ratio in the developing retina.

BETA2/NeuroD1 knockout mice display a 50% reduction in rod-driven electroretinograms (ERGs) and a 65% reduction in cone-driven ERGs at 2–3 mo of age. Neither rod- nor cone-driven ERGs are detectable after 9 mo of age. These results are in consistent with histological analysis, which indicates that *BETA2/ NeuroD1* knockout mice have a reduced number of photoreceptor cells with shortened outer segments of 1–3-mo-old mice. The ONL is completely degenerated after 18 mo of age *(23).* This result suggests that *BETA2/NeuroD1* serves as a cell survival factor. In accordance with this result, studies from cultured retina cells showed that the loss of *BETA2/NeuroD1* resulted in cell death for a subset of photoreceptors, delayed growth of amacrine cells, and an increased number of Müller glial cells *(46).* Beginning at E18.5, there is an increased level of apoptosis in the photoreceptor area in *BETA2/NeuroD1* knockout mice until all photoreceptor cells are completely depleted by 18 mo. The apoptosis in the *BETA2/NeuroD1* knockout retina peaks around P3 in the neuroblastic layer where photoreceptor progenitor cells are localized. We propose that the high level of apoptosis in the *BETA2/NeuroD1* knockout mice is likely due to the synaptic abnormalities between photoreceptors in the ONL and cells in the INL *(23).* Although a single mutation of the *BETA2/NeuroD1* gene was not sufficient to block neurogenesis of cells other than photoreceptor cells, amacrine cells differentiated normally but adopted the ganglion and glial cell fate in double-mutation of *BETA2/NeuroD1* and *Math3 (57).* These studies revealed that *BETA2/NeuroD1* plays an important role in the neuronal subtype specification and terminal differentiation of photoreceptors, and is required for photoreceptor survival.

Inner Ear

The mammalian inner ear contains two major compartments, the cochlea and the vestibule. These organs are responsible for hearing and balance, respectively. The cochlea and vestibule have many hair cells in their epithelium, which play important roles in transducing sound and head movement to the brain as mechanoreceptors. *BETA2/NeuroD1* is first detectable in the ear anlage at the optic cup stage, around E8.5. Prominent expression of *BETA2/NeuroD1* can be observed around E9 in the ventral part of the otic vesicle, where the future cochlear-vestibular ganglion (CVG) and cochlea will be localized. Therefore, *BETA2/NeuroD1* expression precedes the development of these structures and persists in the CVG throughout the development of the inner ear. Starting around E14, *BETA2/NeuroD1* begins to be expressed in the vestibular sensory epithelium and persists until hair-cell differentiation is completed. Low-level expression of *BETA2/NeuroD* was observed in the cochlear sensory epithelium (organ of Corti) after E15.5 *(21).*

As shown in several studies, *BETA2/NeuroD1* knockout mice exhibit several behavioral problems, such as hyperactivity, circling, and lack of righting reflex *(19–21).* The circling behavior is characteristic of mice with defect in the vestibular system, which is important for

balance *(58).* Therefore, the lack of righting reflex and abnormal balance behavior of *BETA2/NeuroD1* knockout mice indicate that these mutants may have defects not only in cerebellar motor function but also in the vestibule system of the inner ear. Histological analysis of *BETA2/NeuroD1* knockout mice showed that *BETA2/NeuroD1* mutants fail to maintain the population of vestibular ganglion due to increased apoptosis *(21,22).* In addition to the balance defect, *BETA2/NeuroD1* mutants also have defects in the cochlear system. A study of auditory brainstem-evoked responses (ABRs), a validation method for hearing, revealed that *BETA2/NeuroD1* knockout mice are deaf *(21).* Histological analysis indicated that mutant mice have a shortened cochlear duct and defects in sensory epithelia of the inner ear. In addition, similar to the vestibular ganglion, the number of cochlear ganglion neurons decreases with age through increased apoptosis. By P7, more than 90% of cochlear ganglion neurons have been lost.

In accordance with this phenotype, the expression of neurotrophin receptors, *TrkB* and *TrkC,* is decreased drastically or is undetectable in the CVG of *BETA2/NeuroD1* knockout mice, while their respective ligands, brain-derived neurotrophic factor *(BDNF)* and neurotrophin-3 *(NT3)* are not affected *(21,22).* Some of the *BETA2/NeuroD1*-expressing neurons are likely proliferative since they are also labeled by BrdU, but the proliferating neuronal cell number is unchanged in *BETA2/NeuroD1* knockout mice as compared to wild-type *(21).* These results suggest that *BETA2/NeuroD1* plays an important role in the inner ear as a differentiation factor and a survival factor by directly and/or indirectly interacting with neurotrophin factors.

Olfactory System

Since *BETA2/NeuroD1* is expressed in the developing and adult olfactory bulb and olfactory epithelium, we are currently investigating the roles of *BETA2/NeuroD1* on olfactory neuronal cell development. At P1, *BETA2/NeuroD1* expression has been detected in the olfactory bulb by several histological methods. This olfactory expression declines gradually to a moderate level during the early postnatal period *(35).* In the developing and adult olfactory bulb, *BETA2/NeuroD1* expression was observed mainly in granule cells of the mitral cell layer, where differentiated olfactory neurons are located, and in the ventricular zone of the olfactory bulb, where premigratory olfactory neurons or olfactory interneurons are distributed. Low-level expression of *BETA2/NeuroD1* was observed in the rostral mitgratory stream (RMS), a structure containing neural progenitors that give rise to olfactory bulb interneurons (J.-H. Cho and M.-J. Tsai, unpublished data). In the developing olfactory epithelium, *BETA2/NeuroD1* transcripts can first be detected at E10 in one to two cell layers above the basement membrane *(35,59). BETA2/NeuroD1* expression in this layer begins to expand to the whole layer of olfactory epithelium and is significantly decreased in the olfactory epithelium of adult mice *(59).* Interestingly, most BrdU-incorporating cells in the basal part of the olfactory epithelium also expressed *BETA2/NeuroD1,* but BrdU-incorporating cells in the nasal cavity part did not express *BETA2/NeuroD1.* The number of the *BETA2/NeuroD1*-expressing cells that also incorporated BrdU gradually decreased as the animal got older *(59).*

The adult olfactory bulb and olfactory epithelium of *BETA2/NeuroD1* knockout mice did not show any difference in size or morphology when compared to the wild-type (J.-H. Cho and M.-J. Tsai, unpublished data). Cell fate determination and differentiation of olfactory sensory neurons is controlled predominantly by *Mash1* and *Neurogenin1,* respectively *(60).* Following cell fate determination there are two parallel regulatory cascades for olfactory sensory neuron differentiation. *BETA2/NeuroD1* is involved in one branch of the olfactory neuron regulatory cascade downstream of *Neurogenin1,* which is regulated by *Mash1.* When *BETA2/NeuroD1* was mutated, the other parallel regulatory pathway, which

is composed of early B cell factor 1 *(Ebf1)* and lim-homeobox gene 1 *(Lhx1),* may compensate for the lack of *BETA2/NeuroD1 (60).* In terms of the neuronal cell maintenance function of *BETA2/ NeuroD1* as shown in retina *(23),* we have found an increased number of apoptotic cells in the olfactory epithelium of *BETA2/NeuroD1* mutants (J.-H. Cho and M.-J. Tsai, unpublished data). But in comparison to the *Mash1* mutant *(61),* the number of apoptotic cells in the *BETA2/NeuroD1* mutant olfactory epithelium much lower. These results imply that gene(s) that have a redundant function as *BETA2/NeuroD1* may also expressed in olfactory sensory neuronal cells.

Summary

Development of the nervous system is achieved by a progressive and stepwise expression of certain sets of genes. Tissue-specific bHLH transcription factors, including *BETA2/NeuroD1,* have been shown to play a pivotal role in this developmental process. In this review, we have examined the importance of the *BETA2/NeuroD1* gene to the development of the nervous system. As a neuronal differentiation factor, *BETA2/NeuroD1* is required for differentiation of cells committed to the neuronal fate during the late stages of neurogenesis. Therefore, it must be working downstream of the determination factors such as members of the *neurogenin* family. Indeed *BETA2/NeuroD1* is transcriptionally regulated by all three members of this family. However, little is known about heterodimerizing partners and target genes of *BETA2/NeuroD1,* which are needed to establish the signal pathway of *BETA2/NeuroD1* function. As for the morphogenesis of the nervous system, *BETA2/ NeuroD1* is prominently expressed in the differentiation zone of the developing nervous system, as shown in Table 1. This implies that *BETA2/NeuroD1* promotes certain cell types, which are under the control of neurogenic determination factors, into fully differentiated neurons. In addition, the function of *BETA2/ NeuroD1* is not limited to the differentiation and morphogenesis of the nervous system; *BETA2/NeuroD1* plays an important role in the maintenance of the nervous system, as a survival factor most likely by influencing the neurotrophin pathways. Further studies are needed to address the mechanisms of the *BETA2/NeuroD1* function on the development of the nervous system.

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