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The Role of Transcription Factor Pitx3 in Dopamine Neuron Development and Parkinson's Disease

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Abstract

Parkinson's disease (PD) is characterized by the selective loss of dopamine (DA) neurons in the substantia nigra compacta (SNc). The transcription factor Pitx3 is important for the differentiation and maintenance of midbrain DA neurons during development. There is highly restricted and constitutive expression of Pitx3 in the SNc and ventral tegmental area (VTA) of the midbrain after birth. In addition to its importance during development, Pitx3 also has roles in the long-term survival and maintenance of the midbrain DA neurons. In this review, we discuss the function of Pitx3 throughout the life of midbrain neurons and the contribution of Pitx3 to disease mechanisms.

Keywords

Mesostriatal; substantia nigra; pituitary homeobox; transcription factor 3; Rieg1; parkinsonian; Nurrl; aphakia

1. INTRODUCTION

1.1. Pitx3 Genomic Structure

Pitx3 (paired-like homeodomain transcription factor 3 or pituitary homeobox 3), which belongs to a pituitary homeobox subfamily, is a homeodomain-containing transcription factor. Among the pituitary homeobox family members, Pitx1 (originally called Ptx1) was the first to be cloned. It was determined to contribute to pituitary development and function [1]. Shortly after a second family member, Pitx2 (called Ptx2 or Rieg1), was cloned and was found to participate in Rieger's syndrome, which is a malformation of craniofacial features [2]. Then, Pitx3 (Plx3) was identified [3,4], and was found to be transiently expressed in skeleton muscle and eye lens during embryogenesis. After birth, Pitx3 has highly restricted and constitutive expression in the SNc and VTA of the midbrain [3]. In the 6-hydroxydopamine (6-OHDA) lesion-induced mouse model of PD and in PD patients, the number of Pitx3-positive neurons is reduced, which correlates with the loss of midbrain DA neurons. This evidence suggests that the Pitx3 gene may play a role in vertebrate development and/or maintenance of midbrain dopamine (DA) neurons.

The Pitx3 gene in mouse is found on Chromosome 19, and it contains four exons. The cDNA is 1379 bp in length. The protein in mice is 302 amino acids in length and has 98% identity with humans. In humans, Pitx3 is located on Chromosome 10, and it also consists of four exons. The cDNA is 1407 bp in length, and the protein is also 302 amino acids in length, as shown in the structure of the human Pitx3 gene (Fig. 1).

1.2. Pitx3 Protein

In human and mouse, the Pitx3 protein consists of 302 amino acids with 98% amino acid identity. The protein is a homeobox transcription factor that plays a key role in coordinating the gene activity that directs cell fate in the development in a wide range of organisms ranging from yeasts to mammals. At the C-terminal, the protein contains an OAR domain, which is found in several paired-like homeodomains [4]. Although the role of OAR in the Pitx3 protein is unknown, in the Cartl protein OAR seems to restrain Cartl protein transcriptional activity through its effect on DNA binding. It is likely that intramolecular interactions between the OAR domain and the N-terminus in the Pitx and Prx OAR-related proteins lead to a protein conformation associated with a relatively inactive state of these transcription factors. In that case, the OAR domain serves as an intramolecular switch [5].

2. EXPRESSION IN MIDBRAIN DOPAMINE NEURONS

2.1. Development of Midbrain Dopamine Neurons

Although DA neurons are localized in the diencephalon and olfactory bulb, the major DA source in the mammalian central nervous system arises from midbrain DA neurons located in the SNc and VTA [6,7]. In adult brain, about 75% of the DA neurons are located in the midbrain [8]. Three main pathways comprise the midbrain dopaminergic systems. The mesostriatal (nigro-striatal) pathway arises from the SNc and projects mainly to the caudate-putamen. This pathway plays an essential role in the control of voluntary movements. Parkinson's disease primarily results from the loss of SNc DA neurons, which especially decreases the DA concentration in the dorsal striatum leading to movement disorders: resting tremor, bradykinesia, postural instability, and rigidity. The mesolimbic pathway arises mainly from the VTA and projects particularly to the ventral striatum, including the nucleus accumbens and olfactory tubercle. Thirdly, the mesocortical pathway also arises mainly from the VTA, but projects particularly to the prefrontal, cingulate, and perirhinal cortex. These last two pathways play important roles in reward and motivation [9].

During development, the precursors of DA neurons migrate from the neuroepithelium to the ventral midbrain. Two diffusible factors, sonic hedgehog (Shh) and fibroblast growth factor (Fgf8), interact to induce neuronal differentiation. Sonic hedgehog, which is secreted by the floor plate [10,11], and the fibroblast growth factor, which is released by the isthmus border region between midbrain and hindbrain, act with other factors to advance development and specification of the precursor cells. The earliest transcription factors for the specification, Lmx1a and Msx1, appear early while the precursor cells are proliferating. These factors are critical for the transition of the precursor cells from proliferation into the early phases of differentiation to midbrain DA neurons [12]. When RNAi is used to silence Lmx1a in chick embryo, DA neurons are lost in the midbrain, and when there is a gain of Lmx1a function in embryonic stem cells, there is a robust generation of DA neurons [13]. The early stage of DA-specific differentiation (at E9–E10 in mice) also involves Lmx1b, the engrailed factors En1 and En2, and Foxa1. At this stage, the first gene for DA synthesis, amino acid decarboxylase (AADC) is induced. In mice, midbrain DA progenitors exit the cell cycle and become post-mitotic between about E9.5 and E13.5 [14]. Subsequently, transcription factors for terminal differentiation are induced: Nurr1 at E10.5 and Pitx3 at E11.5. Nurr1 is required for the induction of tyrosine hydroxylase (TH) at E11.5, vesicular monoamine transporter 2 (Vmat2)

at E12.5, and dopamine transporter (DAT) at E14 [15]. Pitx3 is required for the survival of some terminally differentiating SNc DA neurons [7]. There is likely interaction between Nurr1 and Pitx3, with evidence indicating that Pitx3 releases Nurr1 from a repressed state, thereby, enabling Nurr1 to specifying the dopaminergic phenotype [16].

2.2. Expression Profile of Pitx3 in SNc and VTA

The two main subgroups of DA neurons in the midbrain are the SNc and the VTA. The SNc is located in a more rostral and lateral position, and the VTA is located in a more caudal, ventral, medial position [6,7]. Although both structures contain a high percentage of DA neurons, they project somewhat topologically to generally different targets with some overlap. These wiring differences contribute to their distinct characteristics and functions [7]. In addition, the VTA and SNc have different dependencies on Pitx3. Although Pitx3 is expressed in all midbrain DA neurons, only a subset of the DA neurons is affected in mutant knockout mice lacking Pitx3. This finding suggests Pitx3 has potential differences in expression and function in these two midbrain regions.

In the VTA, Pitx3 and TH are expressed at about the same time, and in the absence of Pitx3 VTA precursors still express TH. However, in the SNc the expression of Pitx3 occurs before TH, and in the absence of Pitx3 the SNc precursors fail to produce TH [17]. Furthermore, in Pitx3-deficient new born mice, VTA DA neurons are intact, but the number of SNc neurons is dramatically decreased and they no longer express TH. Consequently, the projection to the caudate putamen from the SNc is lost [18,19]. These results suggest that Pitx3 has important roles not only in the development of SNc DA neurons, but also for their function and maintenance. The pattern of dopaminergic loss seen in Pitx3 knockout mice is like that seen in patients with Parkinson's disease (PD) suggesting that dysfunction that influences Pitx3 may be a factor in some forms of PD. In addition, the results indicate that studying Pitx3 may shed light on the mechanistic process that may contribute to PD.

2.3. Pitx3 Knock-out Mouse - The Aphakia Mouse

Pitx3 is located close to the aphakia locus on the mouse chromosome 19 [4]. This locus was derived from classical mouse genetics based on an eye defect characterized by the lack of an eye lens - the aphakia homozygous mouse [20]. The genetic analysis of the aphakia mouse revealed a significant deletion in the 3' upstream enhancer and promoter region, exon 1, and part of intron 1 of the Pitx3 gene [21,22]. Therefore, the aphakia mouse includes the Pitx3 gene knock-out. Not surprisingly, therefore, the aphakia mouse did not have detectable Pitx3 expression in midbrain DA neurons [18]. In the aphakia mice, TH positive neurons in the ventral midbrain (i.e., VTA) are like wild-type mice, but from E12.5 onward, the most lateral TH-positive neurons (i.e., SNc) are absent. Indicative of SNc loss, projections from the midbrain to the caudate putamen are absent, whereas the nucleus accumbens and the olfactory tubercle seem normally innervated. Inactivation of the caudate putamen, resulting in lower overall activity levels, is a characteristic of the aphakia mouse. However, there are no overt motor-control defects observed during behavioral testing [18]. This result indicates that the absence of nigro-striatal connections from the onset may be significantly compensated possibly by other DA neurons during development.

3. TARGETS AND REGULATORS OF PITX3

3.1. Pitx3 Regulates TH Expression

TH is the rate-limiting enzyme in the biosynthesis of DA, and it serves as a marker of DA neurons. Its expression begins in the later stages of neuronal maturation. In the SNc, Pitx3 expression is a prerequisite for the subsequent expression of TH, and Pitx3 is required for the continued maintenance of TH expression even in the adult. There is evidence that Pitx3 can

directly bind to the promoter of the TH gene at a bicoid-type binding element (5'-AAAGCC-3'), leading to up regulation [23]. In P19 cells derived from a mouse embryonic carcinoma, Pitx3 activated TH *via* a 5'TAATCC'3 site located in the TH promoter region [24]. The obvious characteristic of Parkinson's disease is the loss of DA signaling, as would occur after the down-regulation of TH in the SNc. It is possible that the DNA binding ability of Pitx3 is influenced by other factors, such as aging, toxin and gene mutation, further suggesting that Pitx3 may be a potential target for PD treatment.

3.2. Pitx3 Regulates Neurotrophic Factors BDNF and GDNF

Neurotrophic factors play a key role in the protection of DA neurons. Brain derived neurotrophic factor (BDNF) and glial cell line derived neurotrophic factor (GDNF) are among the most important neurotrophic factors regulating the differentiation and survival of midbrain DA neurons [25,26]. These factors also are protective of DA neurons against neurotoxins, and reduced BDNF mRNA expression in the SNc has been observed in PD models [27,28].

Over expression of Pitx3 can upregulate the mRNA and protein levels of both BDNF and GDNF in SH-SY5Y cells and primary ventral mesencephalic cultures. Pitx3-transfected astrocytes contain high levels of BDNF and GDNF and significantly protect the rotenone-induced injury of DA neurons [29]. Exogenous administration of GDNF into the striatum alters DA neuron development, increasing the capacity for DA production and release [30]. In return, SNc DA neurons secrete and transport BDNF to striatal neurons, where it regulates striatal D3 receptor expression [31]. Though the detailed mechanism of how Pitx3 promotes the expression of BDNF and GDNF remains unknown, the interactions among these proteins raises the possibility that Pitx3 may provide a point of entry for therapies aimed at enhancing neurotrophic factors to combat the advance of PD.

3.3. Pitx3's Downstream Target Aldehyde Dehydrogenase 2

Aldehyde dehydrogenase 2 (Ahd2, Raldh1) is expressed in the ventral SNc and VTA, the same neurons that are lost in the Pitx3 mutant aphakia mice. Retinoic acid (RA) has a role in the terminal differentiation of this specific set of neurons. Ahd2 is present in the ventricular zone cells. Its transcription is terminated when cells leave the ventricular zone, and under the control of Pitx3, Ahd2 is transcribed again in a subset of DA neurons. There Ahd2 is essential for the synthesis of RA outside the retina. Transgenic expression of Pitx3 in ES cells increases the proportion of specific Ahd2⁺ neurons among DA neurons after *in vitro* differentiation. Ahd2 is under the control of Pitx3, which binds to the promoter of the Ahd2 gene [32]. The number of TH⁺ neurons is significantly increased after RA treatment in the rostral midbrain DA region of Pitx3 lacking (-/-) embryos. This effect is specific for the rostral part of the developing DA area, and is observed exclusively in Pitx3 (-/-) embryos. Although the mechanistic details are not well understood, the requirement for Pitx3 during development can be bypassed by exogenous supplementation of RA to the embryo.

3.4. Pitx3 Regulates Micro-RNA in a Feed-Back Circuit

Micro-RNAs (mi-RNA) are small non-coding transcripts of about 21 nucleotides that are derived from hairpin precursors (pre-miRNA) [33-35]. The nuclear ribonuclease III (RNaseIII) enzyme, Drosha, cleaves the pre-miRNA to yield a 70-100 nucleotide pre-miRNA, which is translocated to the cytoplasm. In the cytoplasm, a second cleavage takes place involving the action of a complex containing another RNase III enzyme, Dicer. Once cleaved, the RNA duplex undergoes unwinding. One strand of the duplex called the guide strand yields an RNA-induced silencing complex (RISC) that acts to silence the target gene. Mi-RNAs are abundant in the nervous system, where they influence development and are likely to be important mediators of plasticity [36]. The mi-RNAs are essential for the terminal differentiation and maintenance of multiple neuron types, including midbrain DA neurons [37].

The expression of one precursor-miRNA, miR-133b, is specifically enriched in the midbrain and deficient in Parkinson's disease patients [37]. MiR-133b is specifically expressed in the midbrain of normal mice, as in humans, but is markedly reduced in rodent DA deficiency models: both the aphakia mouse and the 6-OHDA induced PD mouse. Interestingly, the aphakia mice maintain a population of midbrain DA neurons in the VTA, suggesting that miR-133b is a direct target of Pitx3 transcription activation. Pitx3 can bind to the promoter of miR-133b sequences and up-regulate miR-133b precursor expression [37]. In addition, the Pitx3 3'-untranslated region (3'UTR) was identified as a target of miR-133b activity. The result indicates miR-133b functions within a negative feedback circuit (Fig. 2). Normally, Pitx3 induces transcription of miR-133b, which suppresses Pitx3 expression post-transcriptionally to terminate the developmental signals. In summary, miR-133b regulates the maturation and function of midbrain DA neurons within a negative feedback circuit that includes the paired-like homeodomain transcription factor, Pitx3 [37].

4. PITX3 AND PARKINSON'S DISEASE

4.1. Polymorphisms in Pitx3 may be Associated with PD

Polymorphisms of the Pitx3 gene are associated with sporadic and early onset of PD. One study found an association of PD with the Pitx3 promoter SNP rs3758549: C/T ($p = 0.004$), with the C-allele appearing to be a recessive risk allele with an estimated population frequency of 83% [38]. However, this finding was not confirmed in another study [39], indicating that it is unclear whether the Pitx3 promoter SNP rs3758549 is associated with Parkinson's disease. An allele of the rs4919621 SNP (chr10:103988621) in intron 1 of the Pitx3 gene is significantly more common in PD patients with an early age of onset (≤ 50 years) than in controls or in those with a late age of onset (> 50 years). The C allele of the rs2281983 SNP also is more common in PD patients with an early age of onset than in controls or PD patients with a late age of onset [39]. Although different SNPs were reported by the two groups, there is a tendency indicating that polymorphisms in the Pitx3 gene may influence the susceptibility to PD.

These data indicate that these genetic factors may have a more important role in the early onset than the late onset of PD. Likely because Pitx3 has a role in the survival of DA neurons, it seems that polymorphisms in the Pitx3 gene influence the risk for early degeneration of DA neurons.

4.2. Potential Application of Pitx3 in PD

The potential treatment of PD by cell transplantation has received great attention. The functions of Pitx3 in the development and maintenance of midbrain DA neurons suggest that manipulation of Pitx3 may have a supporting role in such efforts. Furthermore, the cooperative interaction of Nurr1 and Pitx3 offers other potential avenues for inducing embryonic stem cell maturation into the midbrain DA neuron phenotype [40]. For example, over expression of Pitx3 in ventral mesencephalon derived neural progenitor cells improves motor function in the 6-OHDA-lesion animal model of PD [41] and induces adult human mesenchymal stem cells to adopt a dopaminergic phenotype [42].

5. CONCLUSION

As an important transcription factor during DA neuron development, Pitx3 selectively supports SNc DA neuron differentiation and survival. Pitx3 regulates TH expression and maintains the dopaminergic phenotype, and Pitx3 also has the capability of up regulating the neurotrophic factors BDNF and GDNF, which promote DA neuron survival and protect against insults. Through its downstream target Adh2, Pitx3 induces DA neuron differentiation, which is regulated in a negative feedback loop by miR-133b (Fig. 2). The importance of Pitx3 is

exemplified by the findings that polymorphisms in *Pitx3* may be associated with early onset PD in humans. These results taken together suggest that *Pitx3* may be an entry point for manipulating the mechanisms that are crucial for slowing or reversing the degeneration associated with PD. These kinds of mechanisms may serve as ancillary controls for inducing human stem cells to express a dopaminergic phenotype.

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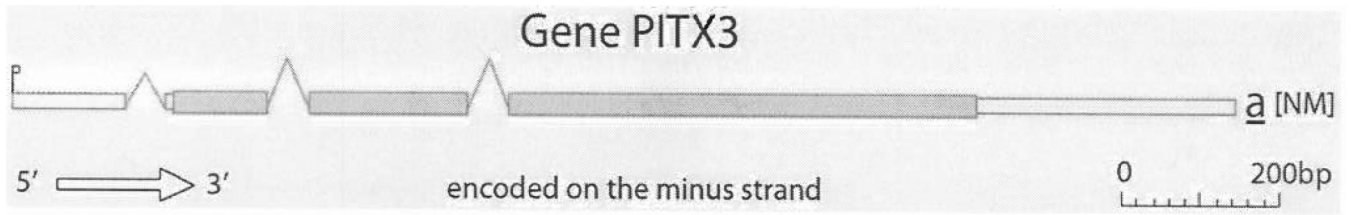


Fig. (1).

Representation of the human Pitx3 gene structure. The gene PITX3 maps on chromosome 10, at 10q25 according to Entrez Gene. Exon 1 is a 5'UTR of 143 bp, Exon 2 is 130 bp, Exon 3 is 203 bp and Exon 4 is 916 bp. As obtained from AceView, the coding region starts from the Exon 2 and extends to Exon 4, as indicated by the shaded regions.

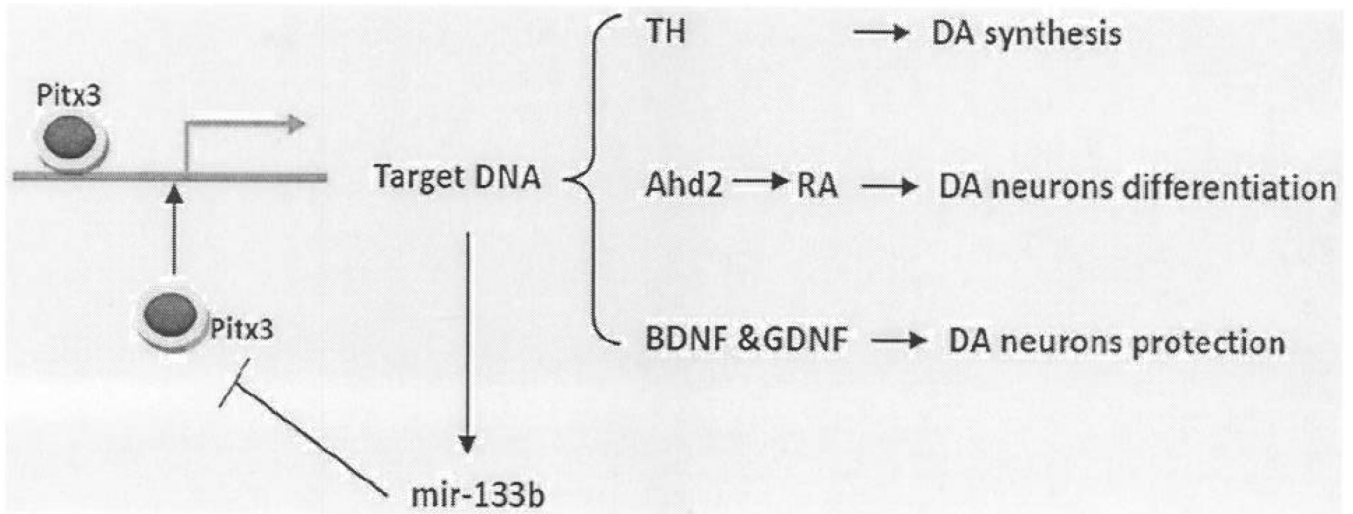


Fig. (2). Summary of the regulation of DA neuronal events in the brain. An auto-regulatory feedback loop onto Pitx3 *via* miR-133b is shown with the downstream targets of Pitx3 in DA neuron maturation.