Nurr1: a new Insight to protects dopaminergic neurodegeneration in Parkinson’s disease

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The developmental transcription factors are important in early neuron specification and differentiation often remains expressed in the adult brain for regulation and maintenances of essential neurophysiological functions. The involvement of transcription factors required for the regulation of long-term survival of central dopaminergic neurons may provide new insight into the etiology and molecular mechanisms leading to dopaminergic cell deaths in Parkinson’s disease (PD). Nurr1, a transcription factor belonging to the orphan nuclear receptor super-family play a vital role in the development, maintenance and survival of dopaminergic neurons. It appears to regulate the expression of dopaminergic markers, and synthesis, transport and storage of dopamine. Decreased Nurr1 expression is found in the autopsied PD midbrains, particularly in neurons containing Lewy bodies, as well as in peripheral lymphocytes of patients with PD. Several variants in Nurr1 gene have been reported in association with PD, in this review we proposed that Nurr1 is an essential factor for the maintenance of dopaminergic neuron functions, but it may also play a pivotal role in the pathogenesis of PD.

Keywords: Nurr1; Parkinson’s disease; Dopaminergic neurons; Tyrosine hydroxylase; Dopamine


Introduction

Parkinson’s disease (PD) is the age-associated second most common neurodegenerative disease after Alzheimer’s disease, characterized by progressive loss of dopaminergic neurons. Most cases of PD will have multi-factorial etiologies, mostly appeared with genetic components. Although predominantly idiopathic, genetic mutations account for ~10% of PD cases [1]. Current findings suggest that genes responsible for PD are yielding critical insights into mechanisms shared by sporadic and familial form. In some rare of PD caused by single-gene [2, 3], but recent research found that individuals with below 50 [4] or 70 [5] years of age are more likely to have major genetic contributions. Approximately 15% of cases have first and second degree relatives with PD [5, 6]. Therefore, hereditary cases comprise the huge dominant in the pathogenesis of PD, because of the largely multi-factorial etiology of PD. Much of its analytical epidemiology will likely be increasingly concerned with defining the intra cellular environmental factors that confer risk or protection. Identifying the genetic factors that modify disease susceptibility and determining the relative roles played by these factors, alone and interactively.

The molecular mechanisms leading to the degeneration of nigral dopaminergic neurons in PD are unclear. Reports are suggested that insufficiency in certain key molecules such as transcription factors can lead to a neurodegenerative phenotype and subsequently to PD-like symptoms [7, 8, 9, 10]. However, to study how these transcription factors function to
maintain appropriate dopaminergic identities in adult brain. How the transcription factor dysregulation may contribute to dopaminergic denervation in substantia nigra (SN) may provide new insights into the etiology and molecular mechanisms leading to midbrain dopaminergic neuronal loss in PD. The Nurr1 gene, a member of nuclear hormone-receptor family \[^{11}\] is an important predominant transcription factor in the brain. It is highly expressed in dopaminergic neurons during development, maintenance throughout and survival in adulthood brain \[^{12,13}\]. In addition, it has been suggested that Nurr1 can be a potential target for the study of novel therapeutic strategies in PD \[^{14}\]. In this review we highlighted and discussed understanding the role of Nurr1, a PD-related gene in dopaminergic neurobiology may provide new insight into the underpinning pathogenic mechanisms of new neuroprotective strategy and neurodegeneration.

**Nuclear-hormone receptors**

The nuclear-hormone receptors (NR) are transcriptional activators that regulate hormone dependent differentiation \[^{15}\] and increase gene activity by recruiting co-activators that assist in chromatin remodeling \[^{16}\]. 48 members of NR superfamily can be subdivided into three major categories, namely, the endocrine nuclear receptors, adopted orphan receptors, and orphan receptors such as NR4A for which cognate ligands have not yet been identified. The NR4A orphan receptor subfamily includes NR4A1 (Nur77, NGFI-B,TR3), NR4A2 (Nurr1, NOT), and NR4A3 (Nor-1, MINOR) \[^{17,18,19,20}\] and it has been suggested that the failure to identify an endogenous ligand may be due to the lack of a typical NR ligand binding pocket in the LBD domain of Nurr1 or NR4A2 receptors \[^{21}\]. Instead, Nurr1 are immediate early genes whose expression is induced by various stimuli including cyclic AMP, growth factors, inflammatory signals, and hormones \[^{17}\].

**Nurr1 gene**

Nurr1 (or NR4A2) member of hormone receptor family was first identified from mouse brain cDNA library in 1992 and localized to human chromosome 2q22-q23 in 1994 \[^{22,23}\]. The DNA sequence of Nurr1 overlaps, especially in the CyS2-CyS2, with that of Nur77 and NOR-1 \[^{21,11}\]. The Nurr1 gene consists with 8 exons and 7 introns; the total length is 9.822 kb. The open reading frame of Nurr1 gene contains 1794 bases that encode for 598 amino acids. The initiation site of translation (initiation codon) is in the third exon and the termination of translation (stop code) is at the upstream region of the eighth exon \[^{24,25}\]. There is a 3’ untranslated region (NTR) is important for the stabilization of mRNA transcription, that contains ATTTA repetitive sequence at the downstream region of the 8 exon and the total length is about 1.3 kb. This feature of Nurr1 gene as an immediate early gene facilitates rapid transcription in response to stimulation by any of several factors involved in the regulation of the gene expression.

NRs are ligand inducible transcription factors that bind to DNA and modulate expression of target genes \[^{26}\]. However, ligands for Nurr1 and several additional orphan receptors remain unknown. The NR super family of transcription factors are characterized by their structural homology which includes N and C-terminal domains (A/B and E/F, respectively), a DNA binding domain (D), and an adjacent hinge region (C), and an adjacent hinge region (D) \[^{27,28,29}\]. Both N- and C terminal regions may contain activation function (AF), and the ligand binding domain (LBD) resides in the C-terminus of NR \[^{30}\]. The N- and C-terminal AF domains including AF-1 and AF-2, respectively, thought to regulate its transcriptional activity \[^{31,32}\].

The DNA binding domain of Nurr1 is highly conserved among the NR family members and is contained of two zinc finger modules. This binding domain is known to activate transcription through binding to an NGFI-B response element (NBRE) \[^{33}\]. Nurr1 lacks a classical binding site for co-activators and the tight packing of side chains from hydrophobic residues in the LBD prevents the molecule from having a ligand binding cavity. The constitutive transcriptional activity of Nurr1, therefore, can be attributed to the canonical protein fold resembling the agonist-bound, transcriptionally active, LBD in NRs \[^{21}\]. Furthermore, Nurr1 also activates transcription through heterodimerization with the retinoid X receptor (RXR) and in response to the RXR ligands \[^{34,35}\]. The AF1 and AF2 domains are also involved in co-factor recruitment and contribute to the constitutive transcriptional activation of Nurr1 \[^{36,37}\].

**Regulation of Nurr1 Expression**

Increasing reports suggested that NR factors transcriptional activity is regulated by primarily at the level of protein expression and post-translational modifications. Nurr1 is primarily regulated by phosphorylation of ERK/AKT, SUMOylation by protein inhibitor of activated STAT-γ, or dimerization with glucocorticoid or retinoid receptors in brain \[^{38}\]. Phosphorylation of NR factors plays an important role in their subcellular translocation and Nurr1 dependent induction of apoptosis \[^{39,40,41}\]. The post-translational modification by SUMOylation has appeared as an important regulatory pathway of Nurr1 transcriptional activity \[^{42,43,44}\]. All NR factors harbor Small ubiquitin-related modifier (SUMO) consensus motifs in their sequences, but only Nurr1 SUMOylation by SUMO2 and
SUMO3. SUMOylation of the N-terminal domain of Nurr1 with SUMO2 reduces its transcriptional activity in promoters harboring more than one NBRE element [44].

In addition, SUMOylation is a reversible process, in which the de-SUMOylation is exerted by SUMO-specific proteases (SENPs) [45]. SUMOylation of transcription factors regulate their half-life, the sub-cellular location and the transcriptional activity, among other features [46, 47]. Interestingly, SUMOylation of several transcription factors as the glucocorticoids, androgen and estrogen nuclear receptors, restricts their transcriptional activity in promoters with several response elements arranged in tandem [48]. This SUMOylation happens on lysines overlapping with a synergy control (SC) motif [49]. Nurr1 is SUMOylated by SUMO-2 at the lysine 91 located in a functional SC motif. Thus, SUMOylation of Nurr1 in the lysine 91 restricts its transcriptional activity in promoters with more than one response element. PIASy increases Nurr1 SUMOylation on lysine 91 and PIASy exert two mechanisms of repression over Nurr1 transitivity, one dependent and other independent of Nurr1 SUMOylation [44].

**Nurr1 and dopaminergic neurons**

The ligand inducible transcription factor Nurr1, is predominately expressed in the CNS in limbic areas and the ventral midbrain, including dopaminergic neurons [50]. Nurr1 expression in the ventral midbrain occurs on embryonic day 10.5 (E10.5), just before the appearance of the dopaminergic marker enzyme tyrosine hydroxylase (TH) at E11.5 [51]. It also, as Nurr1 expression continues throughout adulthood stage, this transcription factor may also regulates normal function of the mature dopaminergic neuronal system. It also seems to play a pivotal role in the migration and striatal target area innervation of differentiating mesencephalic dopaminergic cells [13]. Furthermore, together with other seminal transcription factors such as Pitx3 and Lmx1b, Nurr1 exerts a number of functions in post-mitotic and mature mesencephalic DA neurons, including regulation of TH, dopamine transporter (DAT) and vesicular monoamine transporter2 (VMAT2) expressions [8, 52]. In the absence of Nurr1, developing mesencephalic dopaminergic cells fail to express TH and the receptor TH subunit transfection (Ret) [53]. In contrast, many other dopaminergic markers of DA cells such as Ptx3, Lmx1b, En-1 and En-2 are initially expressed in the absence of Nurr1 but are lost at later stages of development [13]. Moreover, Nurr1-absence in embryonic ventral midbrain cells fails to migrate normally and is unable to innervate their striatal target areas [13]. It has been suggested that Nurr1 deficiency may be a contributing factor in the etiopathogenesis of PD [14].

The reduced Nurr1 expression in mouse models facilitates the emergence of one of the critical neuropathological features of this disorder, namely reduction in the number of dopaminergic cells in the substantia nigra (SN) of PD patients brain [7, 8, 43]. Mutations in NR4A2 and decrease in Nurr1-positive SN neurons have also been shown in PD cases [54, 55, 56], thus further supporting a link between altered Nurr1 expression and PD progression. Recently, two mutations located in exon 1 of the Nurr1 gene were reported in 10 out of 107 individuals with familial PD, and resulted in a marked decrease in Nurr1 mRNA [57]. Moreover, an association of the intronic polymorphism 7048G7049 with both familial PD and sporadic PD was described [58].

**Nurr1 in Microglia and Astrocytes Protects Dopaminergic Neurons from Inflammation-Induced Death**

Neuroinflammatory responses may be impacted by the function of certain gene products in microglia, some of which have thus far been thought to be important only in neurons. Recent work from several groups has begun to elucidate the transcriptional mechanisms through which nuclear receptors repress inflammatory gene expression [59]. Nurr1 mediates the expression of a battery of genes involved in modulation of glial inflammation. The role for Nurr1 in neuroinflammation may provide help in circumventing some of the obstacles that prevent efficacious cell-based therapies for PD. Its expression has been confirmed in microglia and astrocytes and it has been established that inhibits the expression of pro-inflammatory mediators in glial cells, consequently protecting against inflammation-induced dopaminergic neuronal death [60]. It functions as a trans-repressor of pro-inflammatory gene promoters in microglia and astrocytes by recruiting CoREST co-repressor complex similar to the ligand dependent NR PPAR and LXR. Saijo et al. [43] reported that Nurr1 protects dopaminergic neurons by suppressing inflammatory gene expression in astrocytes and microglia, the mechanism by which Nurr1 mediates neuroprotection. Evidences from co-immunoprecipitation experiments that Nurr1-mediated trans-repression involves the physical association of Nurr1 with the p65 subunit of NF-κB. Saijo and his research team [43] investigated the mechanisms underlying the protective effects of Nurr1 via the anti-inflammatory action of PPAR and LXR. The analysis of chromatin immunoprecipitation findings indicates that Nurr1 is entered to the promoters of lipopolysaccharide (LPS) responsive genes. Interestingly, the ability of Nurr1 to inhibit these promoters is signal specific but does not require direct binding to a specific DNA sequence.

In addition, mutations in key phosphorylation sites,
coupled with the use of kinase inhibitors, identify GSK3β-mediated phosphorylation of p65 as a signal for recruitment of Nurr1 to p65. Phosphorylation of serine 468 drives the interaction of Nurr1 and p65, resulting in the attenuation of inflammatory gene transcription by NF-κB. Analogous to PPAR- and LXR-mediated protein-protein interaction so called trans-repression, Nurr1 repression of inflammatory promoters also requires SUMOylation of Nurr1 at key lysine residues \([61, 62]\). This repression provides protection from neuroinflammatory cell death from glial cells action and suggests new potential mechanisms linking Nurr1 function and PD. The authors also reported that by administering LPS to the SN of mice using stereotaxic injections (a technique that uses a coordinate system to precisely target an injection needle to particular regions of the brain). This treatment triggers local inflammation and leads to a loss of neurons expressing TH\(^{1+}\). They demonstrate that inflammation induces Nurr1 expression and that local knockdown of Nurr1 enhances the loss of TH\(^{1+}\) neurons. Interestingly, the primary targets for the neuroprotective effects of Nurr1 appear to be the neighboring microglia and astrocytic cells rather than the neurons themselves. In vitro studies in glial cells implicate these support cells in the release of neurotoxic factors that induce neuronal death.

Furthermore, treatment of cells with the inflammatory cytokine interleukin 1β (IL-1β) promotes Nurr1 SUMOylation. It is also expressed in Nurr1 mRNA is induced by inflammatory stimuli, including LPS, in macrophages \([63]\). Intriguingly, recent observations suggest that Nurr1 can function as repressors of cell type-specific inflammatory responses. The molecular mechanism by which Nurr1 controls transcriptional repression of inflammatory responses and the potential impact of Nurr1 function on the inflammatory component of PD has not been evaluated.

**Association of Nurr1 gene in PD Progression**

Decreased levels of Nurr1 in blood cells, plasma and cerebro-spinal fluid can be demonstrated in PD patients, Nurr1 could be a useful biomarker for PD and related disorders \([64]\). Previously reported that Nurr1 expression as a early biomarker for PD and mutation occur in Nurr1are associated with familial PD, but the underlying basis for this relationship has not been established of the gene are possibly associated with PD. Due to their correlation with major human neurological diseases, dopaminergic neurons are some of the most studied neuronal subtypes. Mesencephalic dopaminergic neurons differentiation requires the activation of a cascade of transcription factors, among which play a crucial role the nuclear receptor. Development of mesencephalic dopaminergic neurons in mice requires the expression of the transcription factor Nurr1. During development the expression of Nurr1 precedes that of Pitx3 and those of typical dopaminergic markers such as TH and DAT that are directly regulated by Nurr1. Loss of Nurr1 functions through gene targeting results in the failure of midbrain progenitors to complete specification of the dopaminergic lineage \([12]\).

The transcriptional activities of Nurr1 evidenced that it is an upstream regulator of genes which is involved in the synthesis, packaging, transport and reuptake of DA in SN \([65, 66]\). It has been shown to enhance the expression of TH by directly trans-activating the promoter of TH \([67]\). Regulation of the DAT gene is another key function of Nurr1 in determining the neurotransmitter phenotype of Mesencephalic dopaminergic neurons. The expression of TH and DAT is regulated by the high affinity binding of Nurr1 to an extended half-hormone response element, NGF1-B responsive element (NBRE), in their 5′-untranslated regions \([68, 69]\). On the other hand, Nurr1 is involved in conversion of L-DOPA to DA and packaging of DA into synaptic vesicles by regulating the expression of aromatic L-amino acid decarboxylase (AADC) and VMAT2, respectively \([66]\).

Nurr1 is also act as a key conversion factor in several survival and maintenance pathways in midbrain dopaminergic neurons. Nurr1 regulates the rearranged in Ret gene from the earliest stages in embryonic development. Ret tyrosine kinase is the high-affinity ligand-binding component of the glial cell line derived neurotrophic factor (GDNF) receptor complex that is attached to the cell surface via a glycosyl phosphatidylinositol anchor \([70]\). GDNF has been shown to protect midbrain dopaminergic neurons against the developmental waves of apoptosis, neurotoxic insults mediated cell death \([71, 72, 73, 74]\). Upon GDNF binding, autophosphorylation of the tyrosine domains of Ret triggers activation of several pathways including PI3K, MAPK which are required for neuronal survival and neurite outgrowth \([75]\). Interestingly, one of the downstream phosphorylation targets of the GDNF signaling pathway is the cAMP response element binding (CREB) protein, which is known to directly regulate the expression of Nurr1 by binding to its promoter \([76]\).

Nurr1 gene contains at least four single nucleotide polymorphisms (SNP) \([58, 57]\). One of the SNP is in the BseR1 restriction site resulting in a homozygous 1347048G7049 in intron 6 (N6P), which shows a significantly higher frequency in familial and sporadic PD \([14, 58]\) and diffuse Lewy body disease \([77]\). Furthermore, variants in Nurr1 gene have been found in association with familial PD \([57]\). Genetic analyses in 201 individuals with PD identified two variants in Nurr1 (-291T del and-245 T→G), mapping to the first
exon of Nurr1 and affect one allele in individuals with familial PD with apparently autosomal dominant form but not in individuals with sporadic PD [57]. Phenotypes of patients with variants in the Nurr1 gene are identical to those of late-onset PD but no pathological data are currently available. These variants are found to decrease Nurr1 mRNA level and affect the transcription of gene that encodes TH. It is postulated that these variants could cause dysfunction of dopaminergic neurons and lead to PD. The variants in Nurr1 in PD seem to be very rare and population-restricted [21, 78, 79, 80]. Recently, two novel variants at exon3 of Nurr1 gene were identified in two non-familial PD patients [81]. The first, a heterozygous C→G transversion at exon3 (253), changes the amino acid serine to cysteine and the second is a223C→T sequence. Either or both of these mutations may affect phosphorylation procedure in transcription of the gene encoding TH. Further studies including haplotype analysis and pathophysiology determination are needed to clarify whether the reported variants are disease causing mutations or susceptible polymorphisms to PD.

Conclusion

The function of Nurr1 in DA neurons is far from established, it is highly essential for the development and maintenance of dopaminergic neurons. Reduced expression of Nurr1 has been linked to the etiopathogenesis of PD. The rapidly emerging evidence that inflammation significantly contributes to the pathology of the disease places neuroinflammation as an important focus of research in PD, and consequently positions immunomodulatory therapies as prime targets for investigation. Specifically, approaches targeting the Nurr1 that proving particularly promising. It is especially encouraging that Nurr1 may have indirect anti-inflammatory effect that ultimately favorable to dopaminergic neuronal survival. Further research on the role of Nurr1 in neuroinflammation will open the way to the development of new therapeutic strategies to prevent the damaging effects of inflammation in PD.

References

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