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Molecular Insights into NR4A2(Nurr1): an Emerging Target for Neuroprotective Therapy Against Neuroinflammation and Neuronal Cell Death

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Abstract

NR4A2 is a nuclear receptor and a transcription factor, with distinctive physiological features. In the cell nuclei of the central nervous system, it is widely expressed and identified as a crucial regulator of dopaminergic (DA) neuronal differentiation, survival, and maintenance. Importantly, it has regulated different genes crucial for dopaminergic signals, and its expression has been diminished in both aged and PD post-mortem brains and reduced in PD patients. In microglia and astrocytes, the expression of NR4A2 has been found where it can be capable of inhibiting the expression of proinflammatory mediators; hence, it protected inflammation-mediated DA neuronal death. In addition, NR4A2 plays neuroprotective role via regulating different signals. However, NR4A2 has been mainly focused on Parkinson's research, but, in recent times, it has been studied in Alzheimer's disease (AD), multiple sclerosis (MS), and stroke. Altered expression of NR4A2 is connected to AD progression, and activation of its may improve cognitive function. It is downregulated in peripheral blood mononuclear cells of MS patients; nonetheless, its role in MS has not been fully clear. miR-145-5p known as a putative regulator of NR4A2 and in a middle cerebral artery occlusion/reperfusion model, anti-miR-145-5p administration promoted neurological outcomes in rat. To date, various activators and modulators of NR4A2 have been discovered and investigated as probable therapeutic drugs in neuroinflammatory and neuronal cell death models. The NR4A2 gene and cell-based therapy are described as promising drug candidates for neurodegenerative diseases. Moreover, microRNA might have a crucial role in neurodegeneration via affecting NR4A2 expression. Herein, we present the role of NR4A2 in neuroinflammation and neuronal cell death focusing on neurodegenerative conditions and display NR4A2 as a promising therapeutic target for the therapy of neuroprotection.

Keywords NR4A2 · Nuclear receptor · Therapeutic target · Neuroinflammation · Neuronal cell death and neuroprotection

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Introduction

The nuclear receptor subfamily 4 group A member 2 (NR4A2), also known as nuclear receptor related 1 protein (Nurr1), NOT, TINUR, or NGFIB, belongs to the group of orphan nuclear receptors with no identified ligand and widely known as a transcription factor with a characteristic physiological role [1, 2]. In the central nervous system (CNS), it is widely expressed particularly in the substantia nigra (SN), ventral tegmental area (VTA), and limbic area [3, 4]. Moreover, it is also expressed in the olfactory bulb, hippocampus, temporal cortex, subiculum, cerebellum, posterior hypothalamus, and habenuclear nuclei [5]. NR4A2 is expressed not only in the CNS but also in other tissues, including the bone, endothelial cells, synovial tissues, adrenal gland, intestine, macrophage, and certain other non-neuronal cells [6]. It is mainly crucial for the differentiation of midbrain

dopaminergic (DA) neurons and its continuous expression being critical to maintaining gene expression in DA neurons [1, 7].

NR4A2 plays a role in the progression of various diseases in the CNS and other organ tissues. It is evident in the pathogenesis of different CNS diseases and disorders, including neuroinflammation [1, 6], Parkinson's disease (PD) [8, 9], Alzheimer's disease (AD) [10], multiple sclerosis (MS) [11], depression [12, 13], and schizophrenia [13, 14]. Moreover, it plays a role in rheumatoid arthritis. It regulates inflammatory processes in synovial cells, and NR4A2 regulates genes involved in synoviocytes, which may play a role in the pathogenesis of rheumatoid arthritis. NR4A2 is elevated in rheumatoid arthritis, leading to increased gene expression of pro-inflammatory genes [15]. Its oncogenic-like role has been reported previously in various instances, such as in facilitation of cell proliferation, survival, transformation, invasion, and migration [2]. In addition, NR4A2 is involved in the progression of various cancers, such as skin cancer, breast cancer, and pancreatic ductal adenocarcinoma [16–18]. For instance, in the prostate cancer tissues, NR4A2 protein expression was higher than benign prostate tissue and silencing of endogenous Nurr1 ameliorated proliferation, migration, and invasion of cells and mediated apoptosis [19].

According to the wider pathological implication, numerous studies have been carried out targeting NR4A2 for the promising therapies for neurodegenerative disease. For example, amodiaquine-treated stimulation of NR4A2 improves cognitive functioning [20]; in a 6-hydroxyl dopamine (6-OHDA)-induced lesion model, SA00025, a novel NR4A2 agonist, produces neuroprotective and anti-inflammatory activities [21]. As NR4A2 is an important transcription factor in the pathogenesis of neurodegenerative diseases (NDDs), through targeting of NR4A2 and its associated receptors and transcription factors, the discovery of therapeutics may be of potential value in treating NDDs. We explore the role of NR4A2 in disease progression, particularly in neuroinflammation and neuronal cell death, and as an emerging target in the therapy of NDDs. In addition, we display general structural features of NR4A2 that are vital for its activation.

Structural Features and Activating Sites of NR4A2

As a group of ligand-regulated transcription factors, the nuclear receptor (NR) superfamily is a group of ligand-regulated transcription factors that control definitive gene activity; therefore, these receptors are significant drug targets [22–24]. In general, NR4A2 is an early gene and numerous stimuli, including cAMP, inflammatory signals, hormones, calcium, and growth factors that can immediately induce its transcriptional activity. By directly acting on the promoters or transcription regulatory elements (i.e., cAMP-response element, CArG-like element, SP-1 element), these modulators can influence NR4A2 expression [25, 26]. As a member of the NR4A subfamily, it shares similar structural features with nuclear receptor subfamily 4 group A member 1 (NR4A1) and nuclear receptor subfamily 4 group A member 3 (NR4A3), including (a) a modulator domain, referred to as the activation function (AF)-1 of the N-terminus, (b) a converse DNA-binding domain (DBD), and (c) a ligand-binding domain (LBD) and its transactivation-dependent AF-2 in the C-terminus [26]. Two zinc fingers of the highly conserved DBD as a monomer or homodimer are able to bind the nerve growth factor-inducible-β-binding response element (NBRE; 5′-AAAGGTCA-3′) or as homodimer attached to the nur-response element (NurRE; 5′-TGACCTTT-n6-AAAGGTCA-3′). During the transcription process, these properties are involved in activating the TH and DAT genes [27, 28]. Moreover, as monomers, homodimers, and heterodimers, NR4A2 or NR4A1 can bind with the retinoid X receptor (RXR). These RXR heterodimers bind to a motif referred to as DR5 which can be competently activated by RXR ligands [29]. Based on the occupation of several bulky hydrophobic residues, the NR4A subfamily does not have an LBD cavity unlike other nuclear receptors [30]. Alternatively, its transcriptional activity appears to be reliant on the AF-1 domain [31]. Therefore, the discovery of compounds is difficult in terms of those that can directly activate NR4A2 through LBD. Besides this, there is the identification of various co-regulator interaction surfaces in the NR4A2 LBD, including residues 592, 593, and 577, notably, the groove between helices 11 and 12, which contributes to the probability of developing NR4A2-activating compounds according to their binding of LBD [32, 33]. Additionally, there are several identified compounds that activate NR4A2 or NR4A1 via their LBDs [34–36]. In general, based on the activated functions of these regions of NR4A2 and numerous studies of NR4A2-activating compounds, it is possible to determine small molecules that can activate NR4A2 through its various domains. Binding site in the NR4A2 for unsaturated fatty acid has recently been identified [37]. Docosahexaenoic acid interacts with NR4A2 LBD with high affinity and also affects NR4A2 transactivation. The general structure of NR4A2 is shown in Fig. 1.

Role of NR4A2 in Neuroinflammation and Neuronal Cell Death: Focus on Neurodegenerative Diseases

Much research has been correlated in NR4A2 into neuroinflammation and neuronal cell death focusing on NDDs. NR4A2 has been known to be implicated in midbrain DA neurons for differentiation, maintenance, and survival [3, 38, 39]. The transcriptional function of NR4A2 regulates several

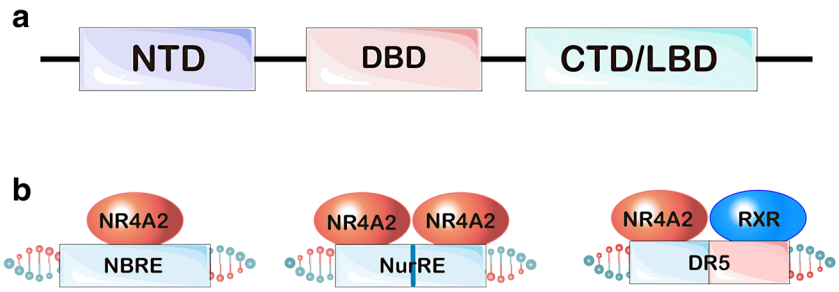

Fig. 1 Basic structure of NR4A2. **a** NR4A2 shares several functional domains, which include DBD and LBD similar to other nuclear receptors. **b** NR4A2 site of actions as monomers at NBRE sites, as dimers at NurRE sites, and as heterodimers with RXR at DR5 sites and activates transcription of many DA-related genes

genes involved in the DA neuronal phenotypes, ranging from DA metabolism, neurotransmission, axonal growth, mitochondrial function, and cell survival [40–44]. Its expression is evident in the embryonic ventral midbrain one day before the appearance of tyrosine hydroxylase (TH, the rate-limiting enzyme in the synthesis of dopamine) [3, 42]. It transcriptionally influences the expression of several phenotypic markers of DA neurons, including TH, dopamine transporter (DAT), L-amino acid decarboxylase (AADC), and vesicular monoamine transporter-2 (VMAT2) [45–47]. The absence of DA neurons in the SN and ventral tegmental area is a consequence of NR4A2 genetic deletion in mice, which may cause their death in case of newborns [39]. Numerous DA neuronal phenotypes, including TH and AADC, are vital in the neurotransmission within the nigrostriatal pathway which are absent in NR4A2-deficient mice [48]. Concerning post-mortem studies, NR4A2 expression is diminished in both aged and PD post-mortem brains [49, 50]. Mutations in NR4A2 have been shown to be associated with familial PD [51], and NR4A2 gene expression is reduced in PD patients [52]. Heterozygous NR4A2 mice survive, displaying reduced dopamine levels in the striatum and midbrain [3] and are more vulnerable to dopaminergic neurotoxins [53]. Experiments using heterozygous NR4A2 knockout mice have recently shown that NR4A2 deficiency impairs dopamine release before the onset of age-related DA neuronal loss [54]. Promising evidence has been proposed via a complicated network between NR4A2 and other crucial transcriptional factors during DA neuronal development. The PITX3 and Wnt/β-catenin pathways are the two major signaling molecules contributing to midbrain DA neurogenesis through cooperation with the NR4A2 transcription complex [38, 55, 56]. Moreover, NR4A2 transcriptionally has controlled many target genes, such as DLK1, PTPRU, KLH1, GTP, and VIP in DA neurons [57–59]. It has been proposed that the involvement of NR4A2 and PITX3 in the expression of novel target genes involved in significant neuronal processes, including neuronal patterning, axon outgrowth, and terminal differentiation, opens up new avenues to study the properties of mdDA neurons during development and in neuronal pathologies, such as experiential in PD [57]. Gene activation by NR4A2 in mdDA neurons and its four major roles are portrayed in Fig. 2.

NR4A2 expression has been established in both microglia and astrocytes [6, 60]. A recent study described how NR4A2 expression may play a significant role in regulating inflammation in the brain [1]. It has been recognized that it inhibits the expression of proinflammatory mediators in these cells, accordingly protects DA neuronal death from inflammation. In adult mice, nigral injection of lentivirus-encoding short hairpin RNA (shRNA) against NR4A2, followed by nigral LPS injection, elevated tumor necrosis factor-alpha (TNF-α) and interleukin 1-beta (IL-1β) levels in microglia and led to a greater reduction of TH-expressing neurons in comparison to LPS treatment alone [60]. The involvement of these proinflammatory cytokines in DA neuronal degeneration has previously been demonstrated in several studies: blockade of the soluble form of the TNF-α receptor attenuated death of DA neurons in rat brains lesioned with 6-hydroxydopamine (6-OHDA), a neurotoxin used to model PD [61]; conditioned medium containing IL-1β and TNF-α from LPS-treated glial cultures induces the death of cultured rat DA neurons [62]; and the chronic expression of IL-1β in adult rat SN using a recombinant adenovirus results in DA neuronal cell death [63]. Employing LPS-treated astrocytic and microglial cultures, it was shown that NR4A2 operates in an anti-inflammatory fashion by docking to target inflammatory gene promoters through association with nuclear factor-kappaB (NF-κB)–p65. NR4A2, then, recruits the CoREST

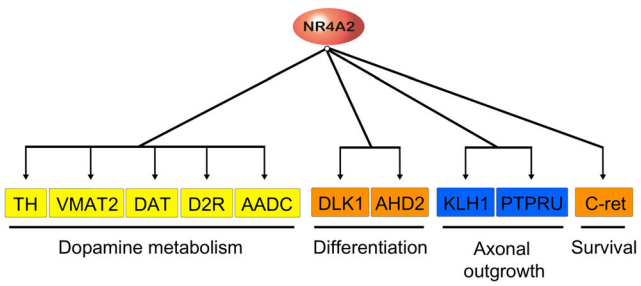

Fig. 2 The activation of different genes in mdDA neurons by NR4A2. The regulations of genes have roles in dopamine metabolism, differentiation, axonal outgrowth, and survival in DA neuron

corepressor complex, restoring the expression of NF-κB-activated genes to basal levels [60]. Moreover, the overexpression of NR4A2-producing neuroprotective and anti-inflammatory activities through downregulating CCL2 in both in vivo and in vitro PD models [64]. According to another recent study, NR4A2 expression in the midbrain of microglia-specific NR4A2 conditional knockout mice was much less than in littermates. An age-dependent decline of rotarod performance was also documented in those mice. The ablation of NR4A2-mediated activation of microglia results in an increase in proinflammatory factor expression. Following LPS stimulation, inflammation injury significantly deteriorated, and DA neuronal loss was exacerbated in NR4A2 conditional knockout mice [65]. NR4A2 has a linkage to MS. However, NR4A2's role in MS is controversial [19, 66]. Considering the gene expression profile, NR4A2 is downregulated in peripheral blood mononuclear cells of MS patients [67]. In an MS murine model, the activation of NR4A2 signaling pathway reduces disease incidence as well as severity through an NF-κB-dependent pathway [11]. Furthermore, in heterozygous NR4A2-knockout mice, chronic experimental autoimmune encephalomyelitis (EAE) courses suggested that NR4A2 is found to be involved in the early phase of MS. This is because NR4A2 defects induce early EAE onset and increase the concentration of inflammatory infiltrates in the spinal cord [68]. NR4A2 is also implicated in stroke, although its involvement is still not fully clear. With this, a rise in NR4A2 expression has therapeutic benefits in the stroke model [69, 70]. By inhibiting the expression of p21 (Waf1/Cip1), NR4A2 specifically promotes intestinal regeneration after ischemia/reperfusion injury [71]. During ischemic stroke, recombinant tissue-type plasminogen activator-treated NR4A2 upregulation is associated with endothelial dysfunction and inflammation as well as enhanced hemorrhagic complications connected to thrombolysis [72].

In addition to the aforementioned, different signaling pathway where NR4A2 is involved in neuroinflammation and neuronal cell death. Phosphorylation of ERK activates NR4A2 [6, 73, 74]. In primary microglial cells, ERK, JNK, or PI3K inhibitors declined NR4A2 expression upon LPS treatment [6]. In addition, phosphorylation of ERK induces NR4A2 expression in neuronal cells [73, 75]. A recent study has correlated phosphorylation of ERK and NR4A2 [76]. Pharmacological treatment, first, caused a rise in ERK phosphorylation, which, then, increased NR4A2 expression, and finally, increased TH, VMAT2, and DAT levels. This study summarized that pharmacological treatment increases TH, DAT, and VMAT2 expressions by upregulating NR4A2 via ERK phosphorylation.

NR4A2 also caused the induction of brain-derived neurotrophic factor (BDNF) transcription [77]. The stimulation of NMDARs promotes neuronal survival during brain development and NR4A2's role in activity-dependent survival of glutamatergic neurons has been reported. In addition, NR4A2 has been found as a downstream target of cAMP response element-binding protein (CREB) and responsible for the NMDA-mediated rise in BDNF, which is necessary for the NMDA-mediated prosurvival effect on neurons [78]. Further, transcription of the glial cell line-derived neurotrophic factor (GDNF) receptor and C-terminal region of the Ret protein (c-Ret) is regulated by NR4A2 [21, 79]. Ret tyrosine kinase is the high-affinity ligand-binding component of the GDNF receptor complex that is attached to the cell surface via a glycosylphosphatidylinositol anchor [79]. GDNF has been shown to protect mesDA neurons against the developmental waves of apoptosis, neurotoxic insult, and cell death. Upon GDNF binding, autophosphorylation of the tyrosine domains of Ret triggers activation of several pathways, including PI3K and MAPK, which are required for neuronal survival and neurite outgrowth [80]. In animals, elevated neuronal degeneration and death owing to be due to mitochondrial dysfunction and an opening of the mitochondrial permeability transition pore [81]. Transcriptional regulation of the pro- and anti-apoptotic members of the B cell lymphoma 2 (Bcl-2) mitochondrial family of proteins, including Bcl-2, B cell lymphoma-extra-large (Bcl-xL), and Bcl-2-associated X protein (Bax) and interaction with the P53 tumor suppressor protein by NR4A2, is responsible for regulation of mitochondrial survival and death. G protein-coupled prostanoid EP1 receptors (EP1) are of concern in the genesis of tumors. One study was conducted to characterize the expression of upregulated NR4A2 by prostaglandin E2 (PGE2)-mediated G protein-coupled prostanoid EP1 receptor stimulation [82]. The EP1 receptor-mediated upregulation of NR4A2 was prevented by the treatment of Rho, PKA, NF-κB, and CREB inhibitors, though PGE2 failed to suggestively stimulate intracellular cAMP formation. PGE2-stimulated EP1 receptor induced the phosphorylation and activation of CREB and NF-κB, which could be blocked by inhibition of PKA. Hence, PGE2-stimulated EP1 receptor upregulates the expression of NR4A2 by a mechanism featuring the sequential activation of the Rho, PKA, CREB, and NF-κB signaling pathways. This upregulation of NR4A2 may underlie the anti-apoptotic effects of PGE2.

NR4A2 features prominently in the brain, particularly in the hippocampus [10, 20]. Subsequent to memory-inducing activities, such as learning and other hippocampus-dependent tasks, NR4A2 expression is upregulated in the hippocampus [83, 84], suggesting NR4A2's involvement in learning and memory. Concerning various studies, lowering NR4A2's level in the hippocampus causes impairments in long-term memory and/or synaptic plasticity [85–88]. A recent study showed that a gradual decrease of NR4A2 concentration in the hippocampus may accompany the normal aging process and a decline in hippocampus-dependent cognitive function [89]. Interestingly, the molecular mechanism

underlying NR4A2 expression in hippocampal and cortical neurons has been demonstrated. Treatment with high KCl and bicuculline upregulated neural activity-enhanced NR4A2, but tetrodotoxin-treated blocking of activity reduced its level. Through voltage-dependent calcium channels and calcineurin, there is a modulation of NR4A2 expression in a cell-autonomous, neural activity-dependent manner [7]. According to one line of experiment, alteration of NR4A2 expression is connected to the progression of AD. With respect to the accumulation of Aβ in 5XFAD mice, NR4A2 is prominently expressed in brain areas such as the subiculum and frontal cortex. This provides information that NR4A2 is highly co-expressed with Aβ during the early stages. Additionally, in an age-dependent fashion, NR4A2-expressing cell numbers suggestively decline in 5XFAD mice, along with increased deposition of plaque [10]. A recent report showed that by elevating adult hippocampal neurogenesis, NR4A2 activation may improve cognitive function [20]. In neural differentiation and axonogenesis, DNA topoisomerase IIβ (topo IIβ) is critical and inhibition of topo IIβ activity leads to shorter axons and increased DNA damage. Moreover, topo IIβ association with NR4A2 has been reported in the onset of AD [90]. Based on topo IIβ deficiency in cultured cerebellar granule neurons and a neurally differentiated human mesenchymal cell line, NR4A2 expression was dramatically downregulated but expression was upregulated in topo IIβ-overexpressing neurally differentiated human mesenchymal cell line.

Role of NR4A2 as a Therapeutic Target for Neuroprotection

NR4A2 Activation in Protecting Against Neurodegeneration

Agonists of NR4A2

Mercaptopurine An anti-leukemia drug, mercaptopurine (6-mercaptopurine or 6-MP) is known as the first identified NR4A2/NR4A3 agonist, which stimulates NR4A2/NR4A3 through directly binding to the N-terminal AF-1 domain [91, 92]. Study has revealed that 6-MP alleviates cerebral infarct in a rodent permanent middle cerebral artery occlusion (pMCAO) model. Besides this, it interrupts the production of IL-1β and TNF-α in CSF and serum [93]. Recent work has demonstrated that 6-MP produces anti-inflammatory responses in LPS-induced inflammation within BV-2 microglia. It significantly attenuates TNF-α production and suppresses transactivation activity of NF-κB and the TNF-α promoter by inhibiting phosphorylation and acetylation of p65 on Ser276 and Lys310, respectively. Following the chromatin immunoprecipitation analyses, 6-MP reduces LPS-induced histone H3 acetylation of chromatin surrounding the TNF-α promoter, ultimately leading to a decrease in p65/coactivator-mediated transcription of the TNF-α gene. Moreover, it enhances NR4A1 expression. Knockdown of NR4A1 expression in 6-MP treated cells results in a significant reduction in 6-MP-mediated inhibition of TNF-α production. In addition, preventing the LPS-activated PI3K/Akt/mTOR signaling cascade, 6-MP delays TNF-α mRNA translation [94].

Daphnane and Phorbol Diterpenes In Korea and mainland China, *Daphne genkwa* Siebold et Zucc. (Family: Thymelaeaceae) is extensively distributed and rather familiar as a traditional oriental medicine [95]. It has wider pharmacological activities, and studies have found that its extract of combined stem and root sample potently activates NR4A2 transcriptional activity at a concentration of 3 μg/mL. Genkwanine N and yuanhuacin are two isolated active compounds found in *D. genkwa*. These daphnane diterpenes significantly improved NR4A2 function at 0.3 μM. On the other hand, NR4A2-specific siRNA abolished the activity of both compounds. Therefore, via modulating the NR4A2 function, both compounds are involved in transcriptional activation. Both compounds also prevented 6-OHDA-induced neuronal cell death and LPS-induced neuroinflammation, respectively. Besides this, these compounds (0.5 mg/kg/day, i.p. for 2 weeks) significantly ameliorated 6-OHDA-induced behavioral deficits and reduced TH-positive DA neuronal death and produced inflammatory responses in rat brains [96]. In addition to yuanhuacin and genkwanine N, a recent study has isolated four more daphnane-type diterpenes (acutilonine F, wikstroemia factor M1, yuanhuadine, and yuanhuatine) and two phorbol-type diterpenes (prostratin Q and 12-O-n-deca-2,4,6-trienoyl-phorbol-(13)-acetate) from the *D. genkwa* extract which have NR4A2-activating properties [97]. Among all the compounds, yuanhuacin, prostratin Q, yuanhuadine, and 12-O-n-deca-2,4,6-trienoyl-phorbol-(13)-acetate displayed higher inhibitory activity in terms of LPS-induced nitric oxide generation in murine microglial BV-2 cells with an IC_{50} (μM) of 1–2, which was 15–30-fold more potent than that of minocycline (29.9 μM). Further, these diterpenes reduced expression and transcription of LPS-induced pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α in BV-2 microglia. A graphical presentation of the mechanism of action of diterpenes from *D. genkwa* is portrayed in Fig. 3.

1,1-Bis (30-Indolyl)-1-(Aromatic) Methane (C-DIM) Analogs According to a number of studies in several cancer cells, 1,1-bis (30-indolyl)-1-(aromatic) methane (C-DIM) analogs are able to influence the expression of the NR4A subfamily [98–101]. The activity of C-DIM against PD has been established in a mouse model. Regarding the activity of different p-substituted phenyl (OCH3, CI, CF3, Br, t-Bu, CN, I, and OCF3), they exhibited similar activating properties,

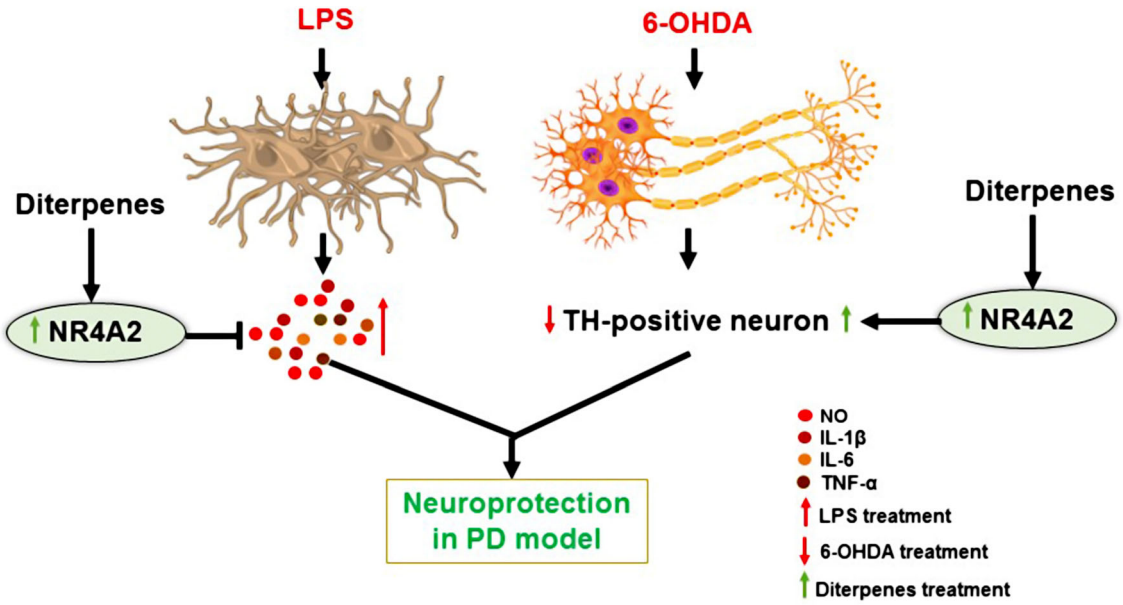

Fig. 3 Protective mechanism of action of diterpenes in PD models via NR4A2. LPS causes inflammation and 6-OHDA reduces number of TH-positive neurons. Treatment of diterpenes increases transcriptional activity of NR4A2, and diterpenes produce protective activity against LPS and 6-OHDA via NR4A2

suggesting a structure–activity relationship (SAR) with these compounds that contain a bis (30-indolyl) moiety [102]. However, both N- and C-terminal domains are involved in NR4A2 activation, but direct binding between C-DIM analogs and NR4A2 is not supported [101]. Moreover, C-DIM5 and CDIM8 have a greater affinity for NR4A1 among the different C-DIM analogs but have opposing effects [98, 100]. In an MPTP-lesioned rat model of PD, C-DIM12 has a higher affinity for NR4A2 and showed the most potent neuroprotective and anti-inflammatory effects among the different C-DIM analogs [103]. It could improve the expressions of both NR4A2- and NR4A2-regulated proteins, including TH and DAT. In addition, C-DIM12 showed NR4A2-mediated recruitment of CoREST and suppression of the NF-κB-mediated inflammatory gene expression in SN [103–105]. A recent study showed that C-DIM 12 suppressed MPTP-induced glial activation and neuronal loss in mice [106]. In vitro reporter analysis also demonstrated that C-DIM12 is an effective activator of NR4A2, and computational modeling has suggested that C-DIM12 has a high affinity to the 3D structure of human NR4A2.

BRF110 BRF110 is a unique in vivo active Nurr1:RXRα-selective lead molecule [107]. It prevents the death of DA neurons and striatal DA denervation in vivo against PD-causing toxins in a NR4A2-dependent manner. It also protects against PD-related genetic mutations in patient-induced pluripotent stem cell (iPSC)-derived DA neurons and a genetic mouse PD model. In addition to neuroprotection, BRF110 upregulates transcription of TH, AADC, and GTP cyclohydrolase I; there is also increased striatal dopamine in vivo. Moreover, chronic daily treatment of BRF110 has symptomatic efficacy without causing dyskinesias in two post-neurodegeneration PD models. The collective neuroprotective and symptomatic effects of BRF110 establish Nurr1:RXRα activation as a probable monotherapeutic method for PD.

Isoxazolo-Pyridinone 7e and Its Analog As an NR4A2 activator, isoxazolo-pyridinone 7e (IP7e) attenuates inflammation and neurodegeneration through inhibiting the NF-κB-dependent process [11]. IP7e might be a probable candidate because of its superb oral bioavailability (95%) along with rapid and extensive brain absorption and distribution [108]. Yet, the NR4A2-activating properties of IP7e have only been confirmed in MS models, not PD models. In an experimental autoimmune encephalomyelitis in mice model, via inhibiting the NF-κB pathway-dependent process, IP7e attenuated inflammation and neurodegeneration in spinal cord [11]. On the other hand, an analog of the isoxazolo-pyridinone derivative, SH1, has been confirmed to be effective in improving behavioral performance in a lactacystin-lesioned PD mouse model. Inhibiting the microglia-mediated neuroinflammation and increasing the DA-specific phenotypes may have involved as the main mechanisms for explaining SH1's pharmacological potential [109].

SA00025 and IRX4204 SA00025 is considered as a novel NR4A2 agonist (EC_{50} 2.5 nM). It exhibits a partial neuroprotective effect in PD models induced by inflammatory stimulant poly(I:C) and 6-OHDA. It can modulate numerous DA target genes, including TH, VMAT, DAT, AADC, and c-Ret in

the SN. In addition, it has anti-inflammatory activity by diminishing the activation of microglia and astrocytes [21]. With this, IRX4204 is a second-generation retinoid X receptor agonist that enhances survival and maintenance of DA neurons in a dose-dependent manner [110]. In an experimental autoimmune encephalomyelitis model, treatment with IRX4204 results in immune modulation and profound attenuation of disease severity. However, the linkage to NR4A2 in MS model was not identified [111]. It can cross the blood-brain barrier and reach the brain at nM concentrations as per bioavailability studies. Administration of IRX4204 though the oral route can activate NR4A2 downstream signaling in the SN and attenuate neurochemical and motor deficits in a rat model of PD. Therefore, it represents a novel, potent, and selective activator of the RXR-NR4A2 signal pathway that promotes DA neuron survival in PD prevention and treatment [110].

Amodiaquine and Chloroquine The antimalarial drugs, amodiaquine (AQ) and chloroquine (CQ), along with a pain-relieving drug, glafenine, have all been identified as a novel group of NR4A2 activators. Through direct physical binding, AQ/CQ (EC_{50}: 20–50 μM) can interact with NR4A2-LBD [34] based on a series of analyses involving Biacore S51 SPR sensor, fluorescence quenching analysis, a radioligand-binding assay by [3H]-CQ, and nuclear magnetic resonance. This finding advances our current understanding of the LBD-binding properties and opens the door for further research and development of NR4A2 agonists. Interestingly, these compounds contain an identical 4-amino-7-chloroquinoline scaffold, which may predict a possible SAR. Both of AQ and CQ enhanced the contrasting dual functions of NR4A2 by further increasing transcriptional activation of mDA-specific genes and enhancing transrepression of neurotoxic proinflammatory gene expression in microglia. In addition, these compounds meaningfully improved behavioral deficits in 6-OHDA lesioned rat models of PD without any noticeable signs of dyskinesia-like behavior [34]. Additionally, the autophagy-regulating effects of AQ/CQ may also predict potential interactions between NR4A2 and autophagy for their anti-parkinsonian effects [112]. AQ-treated activation of NR4A2 may be enhanced by cognitive functions by increasing adult hippocampal neurogenesis displayed by another study [20].

Modulators of NR4A2

Concerning various studies, different compounds, including DA agonists, memantine, retinoic acid-loaded polymeric nanoparticles (RA-NPs), and phyto-bioactive compounds as well as herbal extracts, have been reported to upregulate NR4A2 expression. However, their binding sites to NR4A2 have not been confirmed.

DA Agonists and Memantine

DA agonists neuroprotective action has been considered a controversial issue for decades [113]. They can employ neuroprotection by inducing NR4A2 expression in peripheral blood mononuclear cells as supported by clinical trials [114]. Furthermore, a D2/D3 agonist (pramipexole) has a profound action on improving expression of NR4A2, which precedes the upregulation of both DAT and VMAT2 expression in DA neuronal cell lines [115], signifying that NR4A2 may act as a crucial factor for DA agonist-mediated neuroprotection. Memantine, an N-methyl-D-aspartate receptor antagonist, has been demonstrated to restore PC12 cell survival from 6-OHDA-induced neurotoxicity through upregulating NR4A2 and downregulating NR4A1 along with partially inhibiting migration of NR4A1 from the nucleus to mitochondria [116]. Different from NR4A2, NR4A1 usually triggers the apoptotic process when it migrates to mitochondria and induces inflammation via the NF-κB pathway [117, 118]. Remarkably, DA activator administration could decline NR4A1 expression [119], suggesting that NR4A2 together with the contra-directional coupling of NR4A2/NRAA1 might have a potential therapeutic role against PD pathogenesis.

Dabigatran Etexilate Dabigatran etexilate is the prodrug of dabigatran. It is known as an oral direct thrombin inhibitor and is marketed in Europe and Canada for the prevention of venous thromboembolic events in major orthopedic surgery [120]. Its featuring of the benzimidazole group has been proposed for NR4A2 upregulating properties. In a rotenone-induced PD model, it mitigates neuronal degeneration caused by rotenone and restores striatal dopamine level with motor recovery in rat [121]. It also enhances NR4A2 expression in the SN and increases transcriptional activation of NR4A2-controlled genes, such as TH, VMAT, GDNF, and later receptor gene cRet, which are vital for development and maintenance of DA neurons. In addition, it suppressed thrombin accumulation in the SN. These effects possibly contributed to suppressing neurotoxic proinflammatory cytokines, which was manifested by decreasing the level of NF-κB and TNF-α.

Cilostazol A phosphodiesterase-3 inhibitor, cilostazol, has been shown to produce neuroprotective activity in a recent rotenone-induced rat's model of PD [122]. It upregulates NR4A2 expression, which results in successful preservation of DA neuron functionality and integrity as verified by the marked amelioration of motor performance in behavioral studies, as well as the increased striatal TH content [122]. Besides this, the anti-inflammatory activity of cilostazol as manifested by impeding the global controller of inflammatory signaling pathway, NF-κB, together with its downstream pro-inflammatory cytokines, including TNF-α and IL-1β through

NR4A2 upregulation and glycogen synthase kinase 3 beta inhibition, has been demonstrated. Moreover, an increase in glycogen synthase kinase 3 beta inhibition leads to suppression of downstream apoptotic biomarkers, viz. cytochrome C and caspase-3. Furthermore, it enhances autophagy as depicted by impeding both LC3-II and P62 levels conceivably through the rise in sirtuin 1 levels [122].

Retinoic Acid Nano-Particles Retinoic acid (RA) receptors are highly expressed in DA neurons, and RA can improve the survival and maturation of neuronal cells [123]. A recent advanced novel formulation of nanoparticles is coupled with RA able to transport into cells rapidly to release RA. In an MPTP mouse model of PD, this formulation employs neuroprotection against DA neuronal damage. Further, administration of RA-NP notably elevates PITX3 and NR4A2 expression levels, bolstering development and functional maintenance of DA neurons in PD [124].

Phyto-Bioactive Compounds and Extracts Several bioactive compounds, herbal preparations, and extracts from natural sources have been reported as NR4A2 modulators; they are also effective in the therapy for PD [125–127]. One example is the mori cortex radicis, the root bark of *Morus alba* L. It consists of numerous phytochemicals and has various pharmacological effects [128, 129]. Moracenin D is an isolated compound from mori cortex radices. In a dopamine-induced PD model, it suggestively upregulates NR4A2 expression but downregulates expression of α-synuclein [125]. EGb 761, a standard extract which is prepared from *Ginkgo biloba* leaves [130, 131], has several effects against several neurological disorders, such as AD, PD, and spinocerebellar ataxia type 17 [131–135]. Moreover, EGb 761 activity has been indicated by clinical trials for treating numerous neuropsychiatric diseases [136, 137]. In MPTP-lesioned mice, it produced neuroprotective effect through elevating the expression of a series of DA-related genes, including TH, VMAT2, DAT, and the dopamine D2 receptor (DRD2) in the SN. At the same time, within the SN, it also upregulated transcription factors (PITX3 and NR4A2) where NR4A2 expression was improved by 148% [138]. Bushen Huoxue decoction (BHD) also increases NR4A2 expression. BHD has been indicated to treat craniocerebral diseases [126]. BHD is a two-herb Chinese medicine that alleviates rat's cognitive impairment in a cerebral hypoperfusion model [139]. In a PD model, it also ameliorates behavioral abnormalities and increases cerebral expression and affinity of DRD2 [140]. Besides this, it increases NR4A2 expression at the mRNA level and elevates TH content in the brain. BHD also repairs injured neuron in SN [141]. According to a recent study, the combined herbal extract of Bupleuri Radix, Moutan Cortex Radicis, and Angelica Dahuricae Radix (MABH) produced neuroprotective activity in a PD model. MABH treatment results in recovery from movement deficiencies and also prevents dopamine depletion while protecting DA neurons from degradation in MPTP-induced subacute mice. Moreover, MABH elevates expression of NR4A2 in the SN of mice. Additionally, MABH treatment has impacts on phosphorylation of extracellular signal-regulated kinase protein through increasing NR4A2 protein expression levels and, eventually, TH, VMAT2, and DAT levels [76]. Modulators of NR4A2 along with their major effects are summarized in Table 1.

Advanced Promising Therapeutic Role of NR4A2 Against Neurodegeneration

NR4A2 has played a significant therapeutic role because of its versatility reported in neurodegeneration. As per our discussion, agonist-mediated activation and modulator-mediated upregulation of NR4A2 may have a great potential in therapy of neurodegeneration. Apart from the drug-induced NR4A2 expression, research has focused on NR4A2-based molecular therapy, such as NR4A2 gene- and cell-based therapy, along with miRNA-targeted therapy and novel drug delivery.

Gene- and Cell-Based Therapies

Gene therapy is thought of as a promising therapeutic option for NDDs [142, 143]. As part of gene therapy, NR4A2-based research has remained in the progressive stages for neurodegeneration. For functional nuclear delivery, human NR4A2 has been fused to SUMO, ubiquitin, and the non-toxic N terminus of LFn, as revealed in the SH-SY5Y cell line. Following TH promoter assays, transcriptional activity of TH upon full-length NR4A2 fusion protein HS-LUNN1 has been confirmed and applied to HS-LUNN1 via SHSY5Y cells leading to protection from neurotoxin 6-OHDA-induced cellular degeneration [144]. In a MPTP-lesioned mice model of PD, DA neuronal density and neurotrophic factor expressions are suggestively improved along with suppression of pro-inflammatory cytokine secretion two months after midbrain AAV-NR4A2/Foxa2 injection. One year after injection, NR4A2/Foxa2-mediated cytoprotective effect is detected, signifying these genes' transfection is a fascinating approach for the therapy for PD [145]. Although the outcome of this trial seems exciting, the safety and feasibility of NR4A2 gene therapy still require further verification. First, the long-term effect of constitutive overexpression is not clear. Second, this therapeutic strategy has only been tested in toxin-induced animal models (such as MPTP), but not in transgenic PD models that may mimic the broader pathology of PD. Third, in order to achieve a better therapeutic outcome, the time window of treatment and a controllable regulation of NR4A2 expression are yet to be further explored. Moreover, recent reports show that by choosing the right vectors and promoters, physiological levels and timing of NR4A2 and Foxa2 expression can be

Table 1 Summary of NR4A2 modulators and their key effects

Modulator	Model	Major effects	Reference
Pramipexole	SH-SY5Y cells	Improves NR4A2 expression and upregulates DAT and VMAT2 expressions	[115]
Memantine	6-OHDA-induced PC12 cells	Restores PC12 cell survival from neurotoxicity via upregulating NR4A2 and downregulating NR4A1 as well as partly preventing NR4A1 migration from the nucleus to mitochondria	[116]
Dabigatran etexilate	Rotenone-induced rat model	Protects against rotenone-induced neuronal cell death and inflammation. Restores striatal dopamine level with motor recovery in rat. Also enhances NR4A2 expression in the SN and increases transcriptional activation of NR4A2-controlled genes, such as TH, VMAT, GDNF, and cRet	[121]
Cilostazol	Rotenone-induced rat model	Protects against neuroinflammation and neuronal cell death. Upregulates NR4A2 expression and improves motor performance as well as increases striatal TH content	[122]
Retinoic acid nano-particles	MPTP-induced mouse model	Shows neuroprotection against DA neuronal damage and elevates PITX3 and NR4A2 expression levels	[124]
Moracenin D	Dopamine-induced SH-SY5Y cells	Upregulates NR4A2 expression and downregulates α-synuclein expression	[125]
EGb 761	MPTP-induced mouse model	Demonstrates neuroprotective effect via elevating the expression such as TH, VMAT2, DAT, and DRD2 in the SN. Also attenuates PD-like behavioral abnormalities and upregulates PITX3 and NR4A2	[138]
BHD	6-OHDA-induced rat model	Ameliorates behavioral abnormalities and increases NR4A2 expression at the mRNA level and elevates TH content in the brain. Also repairs injured neuron in SN	[141]
MABH	MPTP-induced mouse model	Shows neuroprotective activity where ameliorates behavioral abnormalities and dopamine depletion. Also induces phosphorylation of extracellular signal-regulated kinase protein via increasing NR4A2 protein expression levels and, eventually, TH, VMAT2, and DAT levels	[76]

replicated in neural stem/precursor cells. Sequential and controlled expression levels of transcription factors Foxa2 and NR4A2 along with CREB signal activation result in fully mature midbrain-type DA neurons with stable phenotype maintenance and improved cell survival [146]. With this, GDNF fails to restore DA neuronal loss caused by α-synuclein toxicity [147]. NR4A2 overexpression may protect DA neurons against α-synuclein [147], and, concerning this concept, AAV-NR4A2 combination delivery with GDNF or neurturin into the midbrain might progress the result of the existing preclinical or clinical trials, and possible serve as a very attractive alternative therapy for PD [148]. Neuronal stem cell therapy focusing on NR4A2 has been considered center stage in research interests. Genetically engineered DA embryonic stem cells that contain NR4A2 genes produce both electrophysiological and behavioral properties expected of SN-derived DA neurons [149]. A recent study has determined that intrastriatal transplantation of lentiviral vector-mediated NR4A2 gene-modified mesenchymal stem cells (MSCs) has notable therapeutic effects in 6-OHDA-induced PD rats [150]. NR4A2 gene-modified MSCs secrete NR4A2 protein in vitro, and NR4A2 gene-modified MSCs transplanted into the striatum survive and migrate in the brain with some differentiation into TH-positive cells, which results in dramatically ameliorating the abnormal behavior of PD rats and increasing the numbers of DA neurons in the SN. The intrastriatal transplantation of NR4A2 gene-modified MSCs produced protective effect on DA neurons which is closely related to the inhibition of activated glial cells and the reduction of inflammatory mediator expressions [150]. Generating NR4A2-positive neuronal stem cells (NSCs) from human wisdom teeth (tNSC) produced a significant recovery from neurologic dysfunction after MCAO treatment [151]. In a very recent study, NR4A2 has played a role in neuroinflammation and differentiation of neural stem cells (NSCs) co-cultured with primary microglia in a transwell co-culture system. NR4A2 protects DA neurons from neuroinflammation insult by limiting the production of neurotoxic mediators by microglia and maintaining the survival of transplanted NSCs [152]. Moreover, the neuroprotective effects of olfactory ensheathing cells (OECs) with the overexpression of NR4A2 and neurogenin 2 (NGN2) in experimental models of PD have also been reported. OECs-NR4A2-NGN2 increased the viability of PC12 cells, while inhibiting oxidative stress and apoptosis, and these effects could be reversed by pre-treatment of k252a, a TrkB receptor inhibitor. The behavioral deficits of PD rats were ameliorated by the transplantation of OECs-NR4A2-NGN2/VMCs [153]. The advanced promising gene- and cell-based therapies of NR4A2 are summarized in Table 2.

MicroRNA-Targeted Therapy and Novel Drug Delivery

In addition to NR4A2 gene therapy and stem cell therapy, microRNA (miRNA) might be potential therapeutic target for neurodegeneration. A number of studies have reported that NR4A2 activity is suppressed by miRNA. In one study, miR-132 was detected in the cAMP signaling pathway and promoted estradiol synthesis via the translational repression of NR4A2 in ovarian granulosa cells [154]. In embryonic stem cells, miR-132 is a vital molecule that negatively regulated DA neuronal differentiation via directly suppressing NR4A2 expression [155]. Upregulating the miR-132 causes a

Table 2 Promising NR4A2 gene-and cell-based therapies for neurodegeneration

Therapy	Model	Key effects	Reference
Functional nuclear delivery of NR4A2	6-OHDA-induced SH-SY5Y cells	Elevates transcriptional activity of TH and protects against neuronal degeneration	[144]
AAV-NR4A2/Foxa2	MPTP-induced mice model	Improves DA neuronal density and neurotrophic factors, suppresses pro-inflammatory cytokine secretion, and protects against cytotoxicity	[145] [146]
Genetically engineered DA embryonic stem cells contained NR4A2 genes	Rat model of PD	Ameliorates electrophysiological and behavioral properties	[149]
Lentiviral vector-mediated NR4A2 gene-modified MSCs	6-OHDA-induced rat model	Ameliorates abnormal behavior of PD rat and increases the numbers of DA neurons in the SN	[150]
Generating NR4A2-positive neuronal stem cells from tNSCs	MCAO surgery-inflicted rats	Significantly recovers neurologic dysfunction after MCAO treatment	[151]
Overexpression of NR4A2	NSCs and microglia	Protects DA neurons from neuroinflammation insults and maintains survival of transplanted NSCs	[152]
Overexpression of NR4A2	MPP^+-induced PC12 and 6-OHDA-induced rat models	OECs-NR4A2-NGN2 increases viability of PC12 cells, inhibits oxidative stress and apoptosis, and ameliorates behavioral deficits	[153]

significant decrease of NR4A2 and BDNF levels in the mesencephalon of affected rats [156]. Following the computational prediction, miRNA recognition element in the 3′UTR of NR4A2 is responsible for miR-145-5p-mediated suppression, and it has been reported that miR-145-5p is a putative regulator of NR4A2. Overexpression of NR4A2 inhibited TNF-α expression in microglia by trans-repression and finally attenuates ischemia/reperfusion-induced inflammatory and

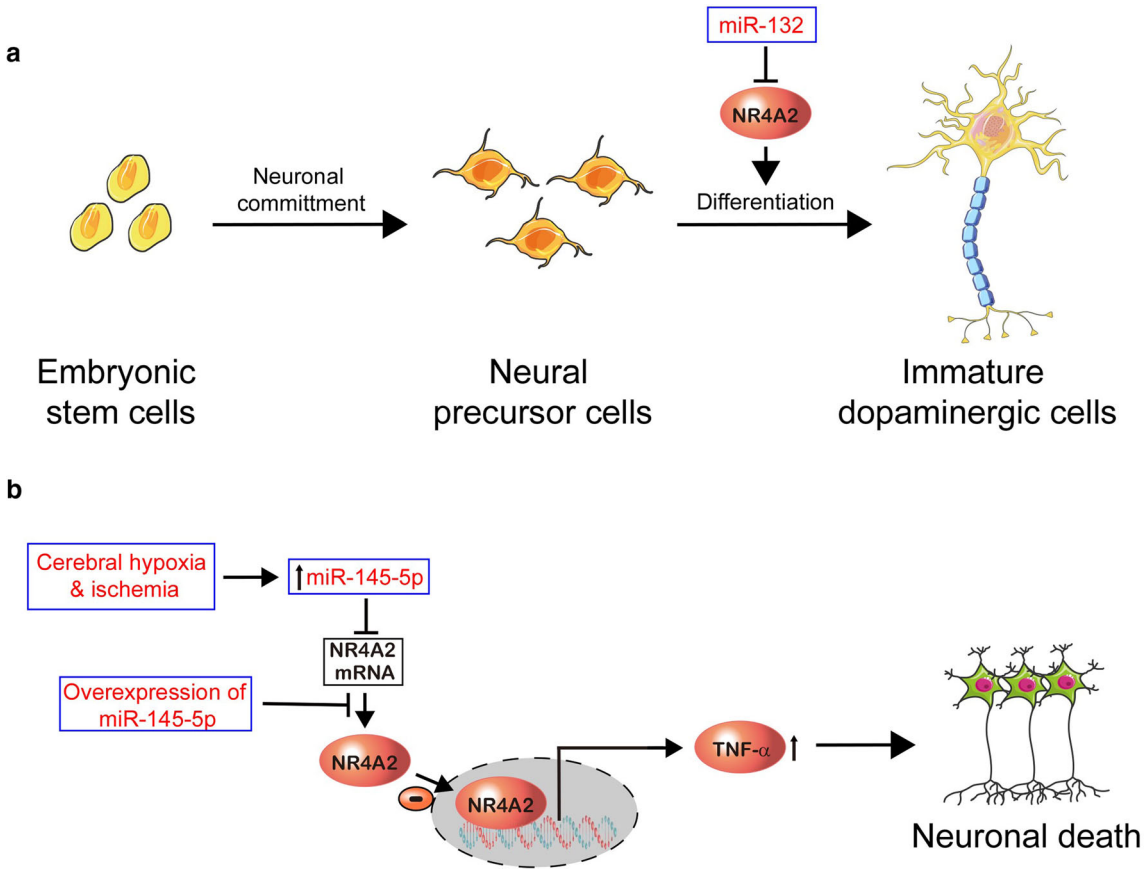

Fig. 4 miRNA as a target for neuroprotection focusing on NR4A2. **a** miR-132 affects differentiation of DA neurons from neuron precursor cells via the suppression of NR4A2. **b** Cerebral hypoxia and ischemia increase miR-145-5p level and overexpressed miR-145-5p prevents NR4A2 functions in microglia. miR-145-5p-mediated suppression of NR4A2 functions, which ultimately causes neuronal cell death via generating TNF-α

cytotoxic response of neurons. In addition, anti-miR-145-5p administration increased expression of NR4A2 and reduced infarct volume in acute cerebral ischemia. Administration of anti-miR-145-5p ameliorated behavioral dysfunction in rat [70]. The role of miRNA as a target for neuroprotection focus on NR4A2 is visualized in Fig. 4.

A research group has recently been demonstrated on novel drug delivery system utilizing NR4A2 [157]. In a 6-OHDA-induced rat model, the delivery of GDNF and NR4A2-polyethylene glycol (PEG)ylated liposomes-coupled microbubbles ameliorated behavioral deficit and increased the TH and DAT immunoreactivity in rat. In addition, this formulation is possibly more efficacious for treating PD than a single treatment following the magnetic resonance imaging (MRI)-guided focused ultrasound analysis [157].

Closing Remarks

Identification of a drug target is a key focus nowadays for the discovery as well as development of therapeutic molecules for treating NDDs. As a transcription factor, NR4A2 is considered crucial to different physiological processes in mammalian organ systems. Considering the accumulating evidence, microglia- and astrocyte-mediated chronic innate neuroinflammation is a common feature across neurodegenerative diseases, including AD, PD, frontotemporal dementia, and amyotrophic lateral sclerosis, and it plays a complex role in their pathophysiology. Neuroinflammation and neuronal cell death are contributed to the pathogenesis of NDDs. Therefore, finding drugs that have potential against neuroinflammation and/or neuronal cell death may be probable candidates for treating NDDs. Herein, we have shown the role of NR4A2, specifically focusing on neuroinflammation and neuronal cell death. As per the overview, it plays a crucial role in the brain and associated physiological processes, especially development, maintenance, and survival of DA neurons, as well as hippocampal normal functioning. Several lines of updated research have reported that NR4A2 has a connection to many receptors and signaling molecules. It regulates many vital transcription factors linked to CNS processes. Focusing on its physiological role in the brain, NR4A2 has been described numerous times in terms of the pathogenesis of different NDDs, such as PD, AD, and MS. Considering this physiological and pathological role, NR4A2-based therapy has been suggested by several research groups. NR4A2 activators and modulators have been investigated as potential therapeutic molecules for the treatment of NDDs in pre-clinical trials. However, many activator- and modulator-binding properties to the NR4A2 LBD are not confirmed, nor is their transcriptional regulation. Therefore, computational and laboratory approaches should be carried out to investigate the agonists and modulator-induced transcriptional regulation of NR4A2. Study should be carried out in knockout and knockdown models to find the neuroprotective role of certain agents via NR4A2-dependent mechanism. Moreover, several analytical techniques, such as biacore S51 SPR sensor, fluorescence quenching analysis, a radioligand-binding assay, and nuclear magnetic resonance, are recommended to elucidate direct binding properties of NR4A2 modulators.

Certain benzimidazole and 4-aminoquinoline groups containing compounds have NR4A2-activating properties. However, these groups featuring many compounds remain to be investigated for their NR4A2 activating and/or modulating properties. In view of the existing data, modification of the available agonists and modulators as well as the discovery of additional NR4A2 activators are proposed. As per the literature, NR4A2 has concentrated mainly on PD, but we provided evidence for its role in other NDDs. Conducting research focused on its pathological and pharmacological roles and its regulation and function in other neurodegenerative conditions, such as AD, MS, and strokes, is highly recommended. Besides this, current challenges for NR4A2-based studies are conducting clinical trial based on available NR4A2 agonists and modulators, along with its associated receptors and signaling molecules. In addition, NR4A2 targeting gene and cell-based therapies are also suggested for clinical trials. We showed that miRNA is a target for protective therapy in our discussion where miR-132 and miR-145p affect neuronal survival via affecting NR4A2. Hence, they may be potential targets for NR4A2 gene and cell-mediated therapy. In conclusion, NR4A2 might have a potential role in the management of NDDs.

Availability of Data and Material Not applicable.

Authors' Contributions MJ, I-SK, and D-KC conceived and designed the study. MJ performed the literature review, wrote the manuscript, and compiled the table. MJ and I-SK also produced the figures, and MEH, SA, and D-YC performed the literature review and data arrangement. D-KC also supervised and handled the correspondence. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Abbreviations 6-OHDA, 6-hydroxyl dopamine; AADC, L-amino acid decarboxylase; AD, Alzheimer's disease; AF-1, Activation function;

CTD, C-terminal domain; CREB, cAMP response element-binding protein; DA, Dopaminergic; DAT, Dopamine transporter; DBD, DNA binding domain; DLK1, Delta-like non-canonical notch ligand 1; GDNF, Glial cell line-derived neurotrophic factor; GTP, Guanosine-5′-triphosphate; IL-1β, Interleukin 1-beta; KLH1, Keyhole limpet hemocyanin1; LBD, Ligand-binding domain; MS, Multiple sclerosis; NTD, N-terminal domain; NDDs, Neurodegenerative diseases; NF-κB, Nuclear factor-kappaB; NGFIB, Nerve growth factor IB; NR4A1, Nuclear receptor subfamily 4 group A member 1; NR4A2, Nuclear receptor subfamily 4 group A member 2; NR4A3, Nuclear receptor subfamily 4 group A member 1; NSCs, Neural stem cells; OECs, Olfactory ensheathing cells; PD, Parkinson's disease; PTPRU, Receptor-type tyrosine-protein phosphatase PCP-2; RXR, Retinoid X receptor; SN, Substantia nigra; TH, Tyrosine hydroxylase; TNF-α, Tumor necrosis factor-alpha; topo IIβ, DNA topoisomerase IIβ; VIP, Vasoactive intestinal peptide; VMAT2, Vesicular monoamine transporter-2

References

1. Lallier SW, Graf AE, Waidyarante GR, Rogers LK (2016) Nurr1 expression is modified by inflammation in microglia. Neuroreport 27(15):1120–1127
2. Beard JA, Tenga A, Hills J, Hoyer JD, Cherian MT, Wang Y-D, Chen T (2016) The orphan nuclear receptor NR4A2 is part of a p53–microRNA-34 network. Sci Rep 6:25108
3. Zetterström RH, Williams R, Perlmann T, Olson L (1996) Cellular expression of the immediate early transcription factors Nurr1 and NGFI-B suggests a gene regulatory role in several brain regions including the nigrostriatal dopamine system. Mol Brain Res 41(1–2):111–120
4. Bäckman C, Perlmann T, Wallén Å, Hoffer BJ, Morales M (1999) A selective group of dopaminergic neurons express Nurr1 in the adult mouse brain. Brain Res 851(1–2):125–132
5. Saucedo-Cardenas O, Conneely OM (1996) Comparative distribution of NURR1 and NUR77 nuclear receptors in the mouse central nervous system. J Mol Neurosci 7(1):51–63
6. Fan X, Luo G, Ming M, Pu P, Li L, Yang D, Le W (2009) Nurr1 expression and its modulation in microglia. Neuroimmunomodulation 16(3):162–170
7. Tokuoka H, Hatanaka T, Metzger D, Ichinose H (2014) Nurr1 expression is regulated by voltage-dependent calcium channels and calcineurin in cultured hippocampal neurons. Neurosci Lett 559:50–55
8. Li T, Yang Z, Li S, Cheng C, Shen B, Le W (2018) Alterations of NURR1 and cytokines in the peripheral blood mononuclear cells: combined biomarkers for Parkinson's disease. Front Aging Neurosci 29(10):392. https://doi.org/10.3389/fnagi.2018.00392
9. Tippabathani J, Nellore J, Radhakrishnan V, Banik S, Kapoor S (2017) Identification of NURR1 (exon 4) and FOXA1 (exon 3) haplotypes associated with mRNA expression levels in peripheral blood lymphocytes of Parkinson's patients in small Indian population. Parkinsons Dis 2017
10. Moon M, Jeong I, Kim CH, Kim J, Lee PK, Mook-Jung I, Leblanc P, Kim KS (2015) Correlation between orphan nuclear receptor Nurr1 expression and amyloid deposition in 5XFAD mice, an animal model of Alzheimer's disease. J Neurochem 132(2):254–262
11. Montarolo F, Raffaele C, Perga S, Martire S, Finardi A, Furlan R, Hintermann S, Bertolotto A (2014) Effects of isoxazolopyridinone 7e, a potent activator of the Nurr1 signaling pathway, on experimental autoimmune encephalomyelitis in mice. PLoS One 9(9):e108791
12. Rojas P, Joodmardi E, Perlmann T, Ögren SO (2010) Rapid increase of Nurr1 mRNA expression in limbic and cortical brain structures related to coping with depression-like behavior in mice. J Neurosci Res 88(10):2284–2293
13. Buervenich S, Carmine A, Arvidsson M, Xiang F, Zhang Z, Sydow O, Jönsson EG, Sedvall GC et al (2000) NURR1 mutations in cases of schizophrenia and manic-depressive disorder. Am J Med Genet A 96(6):808–813
14. Rojas P, Joodmardi E, Hong Y, Perlmann T, Ögren S (2007) Adult mice with reduced Nurr1 expression: an animal model for schizophrenia. Mol Psychiatry 12(8):756–766
15. Davies MR, Harding CJ, Raines S, Tolley K, Parker AE, Downey-Jones M, Needham MR (2005) Nurr1 dependent regulation of pro-inflammatory mediators in immortalised synovial fibroblasts. J Inflamm 2(1):15
16. Ji L, Gong C, Ge L, Song L, Chen F, Jin C, Zhu H, Zhou G (2017) Orphan nuclear receptor Nurr1 as a potential novel marker for progression in human pancreatic ductal adenocarcinoma. Exp Ther Med 13(2):551–559
17. Boakye CH, Doddapaneni R, Shah PP, Patel AR, Godugu C, Safe S, Katiyar SK, Singh M (2013) Chemoprevention of skin cancer with 1, 1-bis (3′-indolyl)-1-(aromatic) methane analog through induction of the orphan nuclear receptor, NR4A2 (Nurr1). PLoS One 8(8):e69519
18. Llopis S, Singleton B, Duplessis T, Carrier L, Rowan B, Williams C (2013) Dichotomous roles for the orphan nuclear receptor NURR1 in breast cancer. BMC Cancer 13(1):139
19. Wang J, Yang J, Zou Y, Huang GL, He ZW (2013) Orphan nuclear receptor nurr1 as a potential novel marker for progression in human prostate cancer. Asian Pac J Cancer Prev 14(3):2023–2028
20. J-i K, Jeon SG, Kim KA, Kim YJ, Song EJ, Choi J, Ahn KJ, Kim C-J et al (2016) The pharmacological stimulation of nurr1 improves cognitive functions via enhancement of adult hippocampal neurogenesis. Stem Cell Res 17(3):534–543
21. Smith GA, Rocha EM, Rooney T, Barneoud P, McLean JR, Beagan J, Osborn T, Coimbra M et al (2015) A Nurr1 agonist causes neuroprotection in a Parkinson's disease lesion model primed with the toll-like receptor 3 dsRNA inflammatory stimulant poly (I: C). PLoS One 10(3):e0121072
22. Chen T (2008) Nuclear receptor drug discovery. Curr Opin Chem Biol 12(4):418–426
23. Gronemeyer H, Gustafsson J-Å, Laudet V (2004) Principles for modulation of the nuclear receptor superfamily. Nat Rev Drug Discov 3(11):950–964
24. Becnel LB, Darlington YF, Ochsner SA, Easton-Marks JR, Watkins CM, McOwiti A, Kankanamge WH, Wise MW et al (2015) Nuclear receptor signaling atlas: opening access to the biology of nuclear receptor signaling pathways. PLoS One 10(9):e0135615
25. Maxwell MA, Muscat GE (2006) The NR4A subgroup: immediate early response genes with pleiotropic physiological roles. Nucl Recept Signal 4:nrs.04002
26. Ichinose H, Ohye T, Suzuki T, Sumi-Ichinose C, Nomura T, Hagino Y, Nagatsu T (1999) Molecular cloning of the human Nurr1 gene: characterization of the human gene and cDNAs. Gene 230(2):233–239
27. Paulsen RE, Granås K, Johnsen H, Rolseth V, Sterri S (1995) Three related brain nuclear receptors, NGFI-B, Nurr1, and NOR-1, as transcriptional activators. J Mol Neurosci 6(4):249–255
28. Maira M, Martens C, Philips A, Drouin J (1999) Heterodimerization between members of the Nur subfamily of orphan nuclear receptors as a novel mechanism for gene activation. Mol Cell Biol 19(11):7549–7557

29. Perlmann T, Jansson L (1995) A novel pathway for vitamin A signaling mediated by RXR heterodimerization with NGFI-B and NURR1. Genes Dev 9(7):769–782
30. Wang Z, Benoit G, Liu J, Prasad S, Aarnisalo P, Liu X, Xu H, Walker NP et al (2003) Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors. Nature 423(6939): 555–560
31. Maira M, Martens C, Batsché É, Gauthier Y, Drouin J (2003) Dimer-specific potentiation of NGFI-B (Nur77) transcriptional activity by the protein kinase A pathway and AF-1-dependent coactivator recruitment. Mol Cell Biol 23(3):763–776
32. Codina A, Benoit G, Gooch JT, Neuhaus D, Perlmann T, Schwabe JW (2004) Identification of a novel co-regulator interaction surface on the ligand binding domain of Nurr1 using NMR footprinting. J Biol Chem 279(51):53338–53345
33. Volakakis N, Malewicz M, Kadkhodai B, Perlmann T, Benoit G (2006) Characterization of the Nurr1 ligand-binding domain coactivator interaction surface. J Mol Endocrinol 37(2):317–326
34. Kim C-H, Han B-S, Moon J, Kim D-J, Shin J, Rajan S, Nguyen QT, Sohn M et al (2015) Nuclear receptor Nurr1 agonists enhance its dual functions and improve behavioral deficits in an animal model of Parkinson's disease. Proc Natl Acad Sci 112(28):8756–8761
35. Zhan Y, Du X, Chen H, Liu J, Zhao B, Huang D, Li G, Xu Q et al (2008) Cytosporone B is an agonist for nuclear orphan receptor Nur77. Nat Chem Biol 4(9):548–556
36. Chintharlapalli S, Burghardt R, Papineni S, Ramaiah S, Yoon K, Safe S (2005) Activation of Nur77 by selected 1, 1-Bis (3′-indolyl)-1-(p-substituted phenyl) methanes induces apoptosis through nuclear pathways. J Biol Chem 280(26):24903–24914
37. de Vera IMS, Giri PK, Munoz-Tello P, Brust R, Fuhrmann J, Matta-Camacho E, Shang J, Campbell S et al (2016) Identification of a binding site for unsaturated fatty acids in the orphan nuclear receptor Nurr1. ACS Chem Biol 11(7):1795–1799
38. Saucedo-Cardenas O, Quintana-Hau JD, Le W-D, Smidt MP, Cox JJ, De Mayo F, Burbach JPH, Conneely OM (1998) Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. Proc Natl Acad Sci 95(7):4013–4018
39. Zetterström RH, Solomin L, Jansson L, Hoffer BJ, Olson L, Perlmann T (1997) Dopamine neuron agenesis in Nurr1-deficient mice. Science 276(5310):248–250
40. Kadkhodaei B, Ito T, Joodmardi E, Mattsson B, Rouillard C, Carta M, Muramatsu S-I, Sumi-Ichinose C et al (2009) Nurr1 is required for maintenance of maturing and adult midbrain dopamine neurons. J Neurosci 29(50):15923–15932
41. Decressac M, Volakakis N, Björklund A, Perlmann T (2013) NURR1 in Parkinson disease—from pathogenesis to therapeutic potential. Nat Rev Neurol 9(11):629–636
42. Kadkhodaei B, Alvarsson A, Schintu N, Ramsköld D, Volakakis N, Joodmardi E, Yoshitake T, Kehr J et al (2013) Transcription factor Nurr1 maintains fiber integrity and nuclear-encoded mitochondrial gene expression in dopamine neurons. Proc Natl Acad Sci 110(6):2360–2365
43. Heng X, Jin G, Zhang X, Yang D, Zhu M, Fu S, Li X, Le W (2012) Nurr1 regulates top IIβ and functions in axon genesis of mesencephalic dopaminergic neurons. Mol Neurodegener 7(1):4
44. Eells JB, Misler JA, Nikodem VM (2006) Reduced tyrosine hydroxylase and GTP cyclohydrolase mRNA expression, tyrosine hydroxylase activity, and associated neurochemical alterations in Nurr1-null heterozygous mice. Brain Res Bull 70(2):186–195
45. Sakurada K, Ohshima-Sakurada M, Palmer TD, Gage FH (1999) Nurr1, an orphan nuclear receptor, is a transcriptional activator of endogenous tyrosine hydroxylase in neural progenitor cells derived from the adult brain. Development 126(18):4017–4026
46. Sacchetti P, Mitchell TR, Granneman JG, Bannon MJ (2001) Nurr1 enhances transcription of the human dopamine transporter gene through a novel mechanism. J Neurochem 76(5):1565–1572
47. Hermanson E, Joseph B, Castro D, Lindqvist E, Aarnisalo P, Wallén Å, Benoit G, Hengerer B et al (2003) Nurr1 regulates dopamine synthesis and storage in MN9D dopamine cells. Exp Cell Res 288(2):324–334
48. Jankovic J, Chen S, Le W (2005) The role of Nurr1 in the development of dopaminergic neurons and Parkinson's disease. Prog Neurobiol 77(1–2):128–138
49. Chu Y, Kompoliti K, Cochran EJ, Mufson EJ, Kordower JH (2002) Age-related decreases in Nurr1 immunoreactivity in the human substantia nigra. J Comp Neurol 450(3):203–214
50. Chu Y, Le W, Kompoliti K, Jankovic J, Mufson EJ, Kordower JH (2006) Nurr1 in Parkinson's disease and related disorders. J Comp Neurol 494(3):495–514
51. Le W-d XP, Jankovic J, Jiang H, Appel SH, Smith RG, Vassilatis DK (2003) Mutations in NR4A2 associated with familial Parkinson disease. Nat Genet 33(1):85
52. Le W, Pan T, Huang M, Xu P, Xie W, Zhu W, Zhang X, Deng H et al (2008) Decreased NURR1 gene expression in patients with Parkinson's disease. J Neurol Sci 273(1):29–33
53. W-d L, Conneely OM, He Y, Jankovic J, Appel SH (1999) Reduced Nurr1 expression increases the vulnerability of mesencephalic dopamine neurons to MPTP-induced injury. J Neurochem 73:2218–2221
54. Zhang L, Le W, Xie W, Dani JA (2012) Age-related changes in dopamine signaling in Nurr1 deficient mice as a model of Parkinson's disease. Neurobiology of Aging 33(5):1001. e1007–1001. e1016
55. Castelo-Branco G, Wagner J, Rodriguez FJ, Kele J, Sousa K, Rawal N, Pasolli HA, Fuchs E et al (2003) Differential regulation of midbrain dopaminergic neuron development by Wnt-1, Wnt-3a, and Wnt-5a. Proc Natl Acad Sci 100(22):12747–12752
56. Joksimovic M, Yun BA, Kittappa R, Anderegg AM, Chang WW, Taketo MM, McKay RD, Awatramani RB (2009) Wnt antagonism of Shh facilitates midbrain floor plate neurogenesis. Nat Neurosci 12(2):125–131
57. Jacobs FM, Van der Linden AJ, Wang Y, von Oerthel L, Sul HS, Burbach JPH, Smidt MP (2009) Identification of Dlk1, Ptpru and Klhl1 as novel Nurr1 target genes in meso-diencephalic dopamine neurons. Development 136(14):2363–2373
58. Gil M, McKinney C, Lee MK, Eells JB, Phyillaier MA, Nikodem VM (2007) Regulation of GTP cyclohydrolase I expression by orphan receptor Nurr1 in cell culture and in vivo. J Neurochem 101(1):142–150
59. Luo Y, Henricksen LA, Giuliano RE, Prifti L, Callahan LM, Federoff HJ (2007) VIP is a transcriptional target of Nurr1 in dopaminergic cells. Exp Neurol 203(1):221–232
60. Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, Gage FH, Glass CK (2009) A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. Cell 137(1):47–59
61. McCoy MK, Martinez TN, Ruhn KA, Szymkowski DE, Smith CG, Botterman BR, Tansey KE, Tansey MG (2006) Blocking soluble tumor necrosis factor signaling with dominant-negative tumor necrosis factor inhibitor attenuates loss of dopaminergic neurons in models of Parkinson's disease. J Neurosci 26(37): 9365–9375
62. Long-Smith CM, Collins L, Toulouse A, Sullivan AM, Nolan YM (2010) Interleukin-1β contributes to dopaminergic neuronal death induced by lipopolysaccharide-stimulated rat glia in vitro. J Neuroimmunol 226(1):20–26
63. Ferrari CC, Godoy MCP, Tarelli R, Chertoff M, Depino AM, Pitossi FJ (2006) Progressive neurodegeneration and motor

disabilities induced by chronic expression of IL-1β in the substantia nigra. Neurobiol Dis 24(1):183–193
64. Liu W, Gao Y, Chang N (2017) Nurr1 overexpression exerts neuroprotective and anti-inflammatory roles via down-regulating CCL2 expression in both in vivo and in vitro Parkinson's disease models. Biochem Biophys Res Commun 482(4):1312–1319
65. Dong J, Wang Y, Liu X, Le W (2018) Nurr1 deficiency-mediated inflammatory injury to nigral dopamine neurons in Parkinson's disease. Parkinsonism Relat Disord 46:e66
66. Satoh J-I, Nakanishi M, Koike F, Miyake S, Yamamoto T, Kawai M, Kikuchi S, Nomura K et al (2005) Microarray analysis identifies an aberrant expression of apoptosis and DNA damage-regulatory genes in multiple sclerosis. Neurobiol Dis 18(3):537–550
67. Gilli F, Lindberg RL, Valentino P, Marnetto F, Malucchi S, Sala A, Capobianco M, di Sapio A et al (2010) Learning from nature: pregnancy changes the expression of inflammation-related genes in patients with multiple sclerosis. PLoS One 5(1):e8962
68. Montarolo F, Perga S, Martire S, Bertolotto A (2015) Nurr1 reduction influences the onset of chronic EAE in mice. Inflamm Res 64(11):841–844
69. Hara K, Matsukawa N, Yasuhara T, Xu L, Yu G, Maki M, Kawase T, Hess DC et al (2007) Transplantation of post-mitotic human neuroteratocarcinoma-overexpressing Nurr1 cells provides therapeutic benefits in experimental stroke: In vitro evidence of expedited neuronal differentiation and GDNF secretion. J Neurosci Res 85(6):1240–1251
70. Xie X, Peng L, Zhu J, Zhou Y, Li L, Chen Y, Yu S, Zhao Y (2017) miR-145-5p/Nurr1/TNF-α signaling-induced microglia activation regulates neuron injury of acute cerebral ischemic/reperfusion in rats. Front Mol Neurosci 10
71. Zu G, Yao J, Ji A, Ning S, Luo F, Li Z, Feng D, Rui Y et al (2017) Nurr1 promotes intestinal regeneration after ischemia/reperfusion injury by inhibiting the expression of p21 (Waf1/Cip1). J Mol Med 95(1):83–95
72. Merino-Zamorano C, Hernández-Guillamon M, Jullienne A, Le Béhot A, Bardou I, Parés M, Fernández-Cadenas I, Giralt D et al (2014) NURR1 involvement in recombinant tissue-type plasminogen activator treatment complications after ischemic stroke. Stroke. https://doi.org/10.1161/STROKEAHA.114.006826
73. Zhang T, Jia N, Fei E, Wang P, Liao Z, Ding L, Yan M, Nukina N et al (2007) Nurr1 is phosphorylated by ERK2 in vitro and its phosphorylation upregulates tyrosine hydroxylase expression in SH-SY5Y cells. Neurosci Lett 423(2):118–122
74. Lu L, Sun X, Liu Y, Zhao H, Zhao S, Yang H (2012) DJ-1 upregulates tyrosine hydroxylase gene expression by activating its transcriptional factor Nurr1 via the ERK1/2 pathway. Int J Biochem Cell Biol 44(1):65–71
75. Lee MK, Nikodem VM (2004) Differential role of ERK in cAMP-induced Nurr1 expression in N2A and C6 cells. Neuroreport 15(1):99–102
76. Sim Y, Park G, Eo H, Huh E, Gu PS, Hong S-P, Pak YK, Oh MS (2017) Protective effects of a herbal extract combination of Bupleurum falcatum, Paeonia suffruticosa, and Angelica dahurica against MPTP-induced neurotoxicity via regulation of nuclear receptor-related 1 protein. Neuroscience 340:166–175
77. Volpicelli F, Caiazzo M, Greco D, Consales C, Leone L, Perrone-Capano C, D'Amato LC, Ud P (2007) Bdnf gene is a downstream target of Nurr1 transcription factor in rat midbrain neurons in vitro. J Neurochem 102(2):441–453
78. Barneda-Zahonero B, Servitja J-M, Badiola N, Miñano-Molina AJ, Fadó R, Saura CA, Rodríguez-Alvarez J (2012) Nurr1 protein is required for N-methyl-D-aspartic acid (NMDA) receptor-mediated neuronal survival. J Biol Chem 287(14):11351–11362
79. Baloh RH, Enomoto H, Johnson EM Jr, Milbrandt J (2000) The GDNF family ligands and receptors—implications for neural development. Curr Opin Neurobiol 10(1):103–110
80. Alavian KN, Jeddi S, Naghipour SI, Nabili P, Licznerski P, Tierney TS (2014) The lifelong maintenance of mesencephalic dopaminergic neurons by Nurr1 and engrailed. J Biomed Sci 21(1):27
81. Imam SZ, Jankovic J, Ali SF, Skinner JT, Xie W, Conneely OM, Le W-D (2005) Nitric oxide mediates increased susceptibility to dopaminergic damage in Nurr1 heterozygous mice. FASEB J 19(11):1441–1450
82. Ji R, Sanchez C, Chou C, Chen X, Woodward D, Regan JW (2012) Prostanoid EP1 receptors mediate up-regulation of the orphan nuclear receptor Nurr1 by cAMP-independent activation of protein kinase A, CREB and NF-κB. Br J Pharmacol 166(3):1033–1046
83. de Ortiz SP, Maldonado-Vlaar CS, Carrasquillo Y (2000) Hippocampal expression of the orphan nuclear receptor gene hzf-3/nurr1 during spatial discrimination learning. Neurobiol Learn Mem 74(2):161–178
84. Vecsey CG, Hawk JD, Lattal KM, Stein JM, Fabian SA, Attner MA, Cabrera SM, McDonough CB et al (2007) Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB: CBP-dependent transcriptional activation. J Neurosci 27(23):6128–6140
85. Colón-Cesario WI, Martínez-Montemayor MM, Morales S, Félix J, Cruz J, Adorno M, Pereira L, Colón N et al (2006) Knockdown of Nurr1 in the rat hippocampus: implications to spatial discrimination learning and memory. Learn Mem 13(6):734–744
86. McQuown SC, Barrett RM, Matheos DP, Post RJ, Rogge GA, Alenghat T, Mullican SE, Jones S et al (2011) HDAC3 is a critical negative regulator of long-term memory formation. J Neurosci 31(2):764–774
87. Hawk JD, Bookout AL, Poplawski SG, Bridi M, Rao AJ, Sulewski ME, Kroener BT, Manglesdorf DJ et al (2012) NR4A nuclear receptors support memory enhancement by histone deacetylase inhibitors. J Clin Invest 122(10):3593–3602
88. Bridi MS, Abel T (2013) The NR4A orphan nuclear receptors mediate transcription-dependent hippocampal synaptic plasticity. Neurobiol Learn Mem 105:151–158
89. Ahn JH, Lee JS, Cho JH, Park JH, Lee TK, Song M, Kim H, Kang SH et al (2018) Age-dependent decrease of Nurr1 protein expression in the gerbil hippocampus. Biomed Rep 8(6):517–522
90. Terzioglu-Usak S, Negis Y, S Karabulut D, Zaim M, Isik S (2017) Cellular model of Alzheimer's disease: A&β1-42 peptide induces amyloid deposition and a decrease in topo isomerase II&β and Nurr1 expression. Curr Alzheimer Res 14(6):636–644
91. Ordentlich P, Yan Y, Zhou S, Heyman RA (2003) Identification of the antineoplastic agent 6-mercaptopurine as an activator of the orphan nuclear hormone receptor Nurr1. J Biol Chem 278(27):24791–24799
92. Wansa KSA, Harris JM, Yan G, Ordentlich P, Muscat GE (2003) The AF-1 domain of the orphan nuclear receptor NOR-1 mediates trans-activation, coactivator recruitment, and activation by the purine anti-metabolite 6-mercaptopurine. J Biol Chem 278(27):24776–24790
93. Chang C-Z, Kwan A-L, Howng S-L (2010) 6-Mercaptopurine exerts an immunomodulatory and neuroprotective effect on permanent focal cerebral occlusion in rats. Acta Neurochir 152(8):1383–1390
94. Huang H-Y, Chang H-F, Tsai M-J, Chen J-S, Wang M-J (2016) 6-Mercaptopurine attenuates tumor necrosis factor-α production in microglia through Nur77-mediated transrepression and PI3K/Akt/mTOR signaling-mediated translational regulation. J Neuroinflammation 13(1):78

95. Van Minh N, Han B-S, Choi H-Y, Byun J, Park J-S, Kim W-G (2017) Genkwalathins A and B, new lathyrane-type diterpenes from Daphne genkwa. Nat Prod Res:1–9
96. Han B-S, Kim K-S, Kim YJ, Jung H-Y, Kang Y-M, Lee K-S, Sohn M-J, Kim C-H et al (2016) Daphnane diterpenes from Daphne genkwa activate Nurr1 and have a neuroprotective effect in an animal model of Parkinson's disease. J Nat Prod 79(6):1604–1609
97. Han B-S, Van Minh N, Choi H-Y, Byun J-S, Kim W-G (2017) Daphnane and phorbol diterpenes, anti-neuroinflammatory compounds with Nurr1 activation from the roots and stems of Daphne genkwa. Biol Pharm Bull 40(12):2205–2211
98. Hedrick E, Lee S-O, Kim G, Abdelrahim M, Jin U-H, Safe S, Abudayyeh A (2015) Nuclear receptor 4A1 (NR4A1) as a drug target for renal cell adenocarcinoma. PLoS One 10(6):e0128308
99. Inamoto T, Papineni S, Chintharlapalli S, Cho S-D, Safe S, Kamat AM (2008) 1, 1-Bis (3′-indolyl)-1-(p-chlorophenyl) methane activates the orphan nuclear receptor Nurr1 and inhibits bladder cancer growth. Mol Cancer Ther 7(12):3825–3833
100. Yoon K, Lee S-O, Cho S-D, Kim K, Khan S, Safe S (2011) Activation of nuclear TR3 (NR4A1) by a diindolylmethane analog induces apoptosis and proapoptotic genes in pancreatic cancer cells and tumors. Carcinogenesis 32(6):836–842
101. Li X, Lee S-O, Safe S (2012) Structure-dependent activation of NR4A2 (Nurr1) by 1, 1-bis (3′-indolyl)-1-(aromatic) methane analogs in pancreatic cancer cells. Biochem Pharmacol 83(10): 1445–1455
102. De Miranda BR, Miller JA, Hansen RJ, Lunghofer PJ, Safe S, Gustafson DL, Colagiovanni D, Tjalkens RB (2013) Neuroprotective efficacy and pharmacokinetic behavior of novel anti-inflammatory para-phenyl substituted diindolylmethanes in a mouse model of Parkinson's disease. J Pharmacol Exp Ther 345(1):125–138
103. De Miranda BR, Popichak KA, Hammond SL, Miller JA, Safe S, Tjalkens RB (2014) Novel para-phenyl substituted diindolylmethanes protect against MPTP neurotoxicity and suppress glial activation in a mouse model of Parkinson's disease. Toxicol Sci 143(2):360–373
104. De Miranda BR, Popichak KA, Hammond SL, Jorgensen BA, Phillips AT, Safe S, Tjalkens RB (2015) The Nurr1 activator 1, 1-bis (3′-indolyl)-1-(p-chlorophenyl) methane blocks inflammatory gene expression in BV-2 microglial cells by inhibiting nuclear factor κB. Mol Pharmacol 87(6):1021–1034
105. Hammond SL, Safe S, Tjalkens RB (2015) A novel synthetic activator of Nurr1 induces dopaminergic gene expression and protects against 6-hydroxydopamine neurotoxicity in vitro. Neurosci Lett 607:83–89
106. Hammond SL, Popichak KA, Li X, Hunt LG, Richman EH, Damale PU, Chong EK, Backos DS et al (2018) The Nurr1 ligand, 1, 1-bis (3′-Indolyl)-1-(p-Chlorophenyl) methane, modulates glial reactivity and is neuroprotective in MPTP-induced parkinsonism. J Pharmacol Exp Ther 365(3):636–651
107. Spathis AD, Asvos X, Ziavra D, Karampelas T, Topouzis S, Cournia Z, Qing X, Alexakos P et al (2017) Nurr1: RXRα heterodimer activation as monotherapy for Parkinson's disease. Proc Natl Acad Sci 114(15):3999–4004
108. Hintermann S, Chiesi M, Von Krosigk U, Mathe D, Felber R, Hengerer B (2007) Identification of a series of highly potent activators of the Nurr1 signaling pathway. Bioorg Med Chem Lett 17(1):193–196
109. Zhang Z, Li X, Xie W-j, Tuo H, Hintermann S, Jankovic J, Le W (2012) Anti-parkinsonian effects of Nurr1 activator in ubiquitin-proteasome system impairment induced animal model of Parkinson's disease. CNS Neurol Disord Drug Targets 11(6): 768–773
110. Wang J, Bi W, Zhao W, Varghese M, Koch RJ, Walker RH, Chandraratna RA, Sanders ME et al (2016) Selective brain penetrable Nurr1 transactivator for treating Parkinson's disease. Oncotarget 7(7):7469–7479
111. Chandraratna RA, Noelle RJ, Nowak EC (2016) Treatment with retinoid X receptor agonist IRX4204 ameliorates experimental autoimmune encephalomyelitis. Am J Transl Res 8(2):1016–1026
112. Qiao S, Tao S, Rojo de la Vega M, Park SL, Vonderfecht AA, Jacobs SL, Zhang DD, Wondrak GT (2013) The antimalarial amodiaquine causes autophagic-lysosomal and proliferative blockade sensitizing human melanoma cells to starvation-and chemotherapy-induced cell death. Autophagy 9(12):2087–2102
113. Blandini F, Armentero M-T (2014) Dopamine receptor agonists for Parkinson's disease. Expert Opin Investig Drugs 23(3):387–410
114. Zhang L-M, Sun C-C, Mo M-S, Cen L, Wei L, Luo F-F, Li Y, Li G-F et al (2015) Dopamine agonists exert Nurr1-inducing effect in peripheral blood mononuclear cells of patients with Parkinson's disease. Chin Med J 128(13):1755–1760
115. Pan T, Xie W, Jankovic J, Le W (2005) Biological effects of pramipexole on dopaminergic neuron-associated genes: relevance to neuroprotection. Neurosci Lett 377(2):106–109
116. Wei X, Gao H, Zou J, Liu X, Chen D, Liao J, Xu Y, Ma L et al (2016) Contra-directional coupling of Nur77 and Nurr1 in neurodegeneration: a novel mechanism for memantine-induced anti-inflammation and anti-mitochondrial impairment. Mol Neurobiol 53(9):5876–5892
117. Kiss B, Tóth K, Sarang Z, Garabuczi É, Szondy Z (2015) Retinoids induce Nur77-dependent apoptosis in mouse thymocytes. Biochim Biophys Acta 1853(3):660–670
118. Li L, Liu Y, H-z C, F-w L, Wu J-f, H-k Z, He J-p, Y-z X et al (2015) Impeding the interaction between Nur77 and p38 reduces LPS-induced inflammation. Nat Chem Biol 11(5):339–346
119. Gervais J, Soghomonian J-J, Richard D, Rouillard C (1999) Dopamine and serotonin interactions in the modulation of the expression of the immediate-early transcription factor, nerve growth factor-inducible B, in the striatum. Neuroscience 91(3): 1045–1054
120. Douxfils J, Buckinx F, Mullier F, Minet V, Rabenda V, Reginster J-Y, Hainaut P, Bruyère O et al (2014) Dabigatran etexilate and risk of myocardial infarction, other cardiovascular events, major bleeding, and all-cause mortality: a systematic review and meta-analysis of randomized controlled trials. J Am Heart Assoc 3(3): e000515
121. Kandil EA, Sayed RH, Ahmed LA, El Fattah MAA, El-Sayeh BM (2017) Modulatory role of Nurr1 activation and thrombin inhibition in the neuroprotective effects of dabigatran etexilate in rotenone-induced parkinson's disease in rats. Mol Neurobiol :1–12
122. Hedya SA, Safar MM, Bahgat AK (2018) Cilostazol mediated Nurr1 and autophagy enhancement: neuroprotective activity in rat rotenone PD model. Mol Neurobiol :1–9
123. Katsuki H, Kurimoto E, Takemori S, Kurauchi Y, Hisatsune A, Isohama Y, Izumi Y, Kume T et al (2009) Retinoic acid receptor stimulation protects midbrain dopaminergic neurons from inflammatory degeneration via BDNF-mediated signaling. J Neurochem 110(2):707–718
124. Esteves M, Cristóvão AC, Saraiva T, Rocha SM, Baltazar G, Ferreira L, Bernardino L (2015) Retinoic acid-loaded polymeric nanoparticles induce neuroprotection in a mouse model for Parkinson's disease. Front Aging Neurosci 7:20
125. Ham A, Lee HJ, Hong SS, Lee D, Mar W (2012) Moracenin D from Mori cortex Radicis protects SH-SY5Y cells against dopamine-induced cell death by regulating Nurr1 and α-synuclein expression. Phytother Res 26(4):620–624
126. Shen W, Luo H, Xu L, Wu Z, Chen H, Liu Y, Yu L, Hu L, Wang B, Luo Y et al (2018). Wnt5a mediates the effects of Bushen Huoxue decoction on the migration of bone marrow mesenchymal stem

cells in vitro. Chin Med 13:45. https://doi.org/10.1186/s13020-018-0200-2
127. Pan T, Xie W, Jankovic J, Le W (2005) Radicicol induces heat-shock protein expression and neuroprotection against rotenone-mediated apoptosis in Sh-sy5y cells. Mov Disord 20:S86
128. Kim H-J, Lee HJ, Jeong S-J, Lee H-J, Kim S-H, Park E-J (2011) Cortex Mori Radicis extract exerts antiasthmatic effects via enhancement of CD4+ CD25+ Foxp3+ regulatory T cells and inhibition of Th2 cytokines in a mouse asthma model. J Ethnopharmacol 138(1):40–46
129. Kim JS, You HJ, Kang HY, Ji GE (2012) Enhancement of the tyrosinase inhibitory activity of Mori cortex radicis extract by biotransformation using Leuconostoc paramesenteroides PR. Biosci Biotechnol Biochem 76(8):1425–1430
130. Bidon C, Lachuer J, Molgo J, Wierinckx A, De La Porte S, Pignol B, Christen Y, Meloni R et al (2009) The extract of Ginkgo biloba EGb 761 reactivates a juvenile profile in the skeletal muscle of sarcopenic rats by transcriptional reprogramming. PLoS One 4(11):e7998
131. Huang D-S, Lin H-Y, Lee-Chen G-J, Hsieh-Li H-M, Wu C-H, Lin J-Y (2016) Treatment with a Ginkgo biloba extract, EGb 761, inhibits excitotoxicity in an animal model of spinocerebellar ataxia type 17. Drug Des Devel Ther 10:723
132. Chandrasekaran K, Mehrabian Z, Spinnewyn B, Drieu K, Fiskum G (2001) Neuroprotective effects of bilobalide, a component of the Ginkgo biloba extract (EGb 761), in gerbil global brain ischemia. Brain Res 922(2):282–292
133. Oyama Y, Chikahisa L, Ueha T, Kanemaru K, Noda K (1996) Ginkgo biloba extract protects brain neurons against oxidative stress induced by hydrogen peroxide. Brain Res 712(2):349–352
134. Bastianetto S, Ramassamy C, Doré S, Christen Y, Poirier J, Quirion R (2000) The ginkgo biloba extract (EGb 761) protects hippocampal neurons against cell death induced by β-amyloid. Eur J Neurosci 12(6):1882–1890
135. Diamond BJ, Shiflett SC, Feiwel N, Matheis RJ, Noskin O, Richards JA, Schoenberger NE (2000) Ginkgo biloba extract: mechanisms and clinical indications. Arch Phys Med Rehabil 81(5):668–678
136. von Gunten A, Schlaefke S, Überla K (2016) Efficacy of Ginkgo biloba extract EGb 761® in dementia with behavioural and psychological symptoms: a systematic review. World J Biol Psychiatry 17(8):622–633
137. Chen X, Hong Y, Zheng P (2015) Efficacy and safety of extract of Ginkgo biloba as an adjunct therapy in chronic schizophrenia: a systematic review of randomized, double-blind, placebo-controlled studies with meta-analysis. Psychiatry Res 228(1): 121–127
138. Rojas P, Ruiz-Sanchez E, Rojas C, Ogren SO (2012) Ginkgo biloba extract (EGb 761) modulates the expression of dopamine-related genes in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in mice. Neuroscience 223:246–257. https://doi.org/10.1016/j.neuroscience.2012.08.004
139. Ye S, Gu Y, Xu Y, Fan W, Wang X, Chen S, Cai S, Lv S et al (2014) Bushen Huoxue decoction improves cognitive decline in rats with cerebral hypoperfusion. Mol Med Rep 10(3):1635–1641
140. Wang H, Yang M, Dou Y, Liu Y, Li S, Li M (2011) Effect of Bushen Huoxue decoction on dopamine D2 receptor in the brain of rats with Parkinson's disease. Nan Fang Yi Ke Da Xue Xue Bao 31(11):1879–1881
141. Yang M-h, Wang H-m, Liu Y (2011) Effect of Bushen Huoxue decoction (补肾活血饮) on the orphan receptor and tyrosine hydroxylase in the brain of rats with Parkinson's disease. Chin J Integr Med 17(1):43–47
142. O'Connor DM, Boulis NM (2015) Gene therapy for neurodegenerative diseases. Trends Mol Med 21(8):504–512
143. Piguet F, Alves S, Cartier N (2017) Clinical gene therapy for neurodegenerative diseases: past, present, and future. Hum Gene Ther 28(11):988–1003
144. Paliga D, Raudzus F, Leppla SH, Heumann R, Neumann S (2018) Lethal factor domain-mediated delivery of Nurr1 transcription factor enhances tyrosine hydroxylase activity and protects from neurotoxin-induced degeneration of dopaminergic cells. Mol Neurobiol :1–11
145. Oh SM, Chang MY, Song JJ, Rhee YH, Joe EH, Lee HS, Yi SH, Lee SH (2015) Combined Nurr1 and Foxa2 roles in the therapy of Parkinson's disease. EMBO Molecular Medicine: e201404610
146. Kim T, Song J-J, Puspita L, Valiulahi P, Shim J-W, Lee S-H (2017) In vitro generation of mature midbrain-type dopamine neurons by adjusting exogenous Nurr1 and Foxa2 expressions to their physiologic patterns. Exp Mol Med 49(3):e300
147. Decressac M, Kadkhodaei B, Mattsson B, Laguna A, Perlmann T, Björklund A (2012) α-Synuclein-induced down-regulation of Nurr1 disrupts GDNF signaling in nigral dopamine neurons. Sci Transl Med 4(163):163ra156
148. Bartus RT, Baumann TL, Siffert J, Herzog CD, Alterman R, Boulis N, Turner DA, Stacy M et al (2013) Safety/feasibility of targeting the substantia nigra with AAV2-neurturin in Parkinson patients. Neurology 80(18):1698–1701
149. Kim J-H, Auerbach JM, Rodríguez-Gómez JA, Velasco I, Gavin D, Lumelsky N, Lee S-H, Nguyen J et al (2002) Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. Nature 418(6893):50–56
150. Wang X, Zhuang W, Fu W, Wang X, Lv E, Li F, Zhou S, Rausch W-D et al (2018) The lentiviral-mediated Nurr1 genetic engineering mesenchymal stem cells protect dopaminergic neurons in a rat model of Parkinson's disease. Am J Transl Res 10(6):1583–1599
151. Yang K-L, Chen M-F, Liao C-H, Pang C-Y, Lin P-Y (2009) A simple and efficient method for generating Nurr1-positive neuronal stem cells from human wisdom teeth (tNSC) and the potential of tNSC for stroke therapy. Cytotherapy 11(5):606–617
152. Chen X, Qian Y, Wang X, Tang Z, Xu J, Lin H, Yang Z, Song X et al (2018) Nurr1 promotes neurogenesis of dopaminergic neuron and represses inflammatory factors in the transwell coculture system of neural stem cells and microglia. CNS Neurosci Ther 24: 790–800
153. Liu Q, Qin Q, Sun H, Zhong D, An R, Tian Y, Chen H, Jin J, Wang H, Li G (2017) Neuroprotective effect of olfactory ensheathing cells co-transfected with Nurr1 and Ngn2 in both in vitro and in vivo models of Parkinson's disease. Life Sci
154. Wu S, Sun H, Zhang Q, Jiang Y, Fang T, Cui I, Yan G, Hu Y (2015) MicroRNA-132 promotes estradiol synthesis in ovarian granulosa cells via translational repression of Nurr1. Reprod Biol Endocrinol 13(1):94
155. Yang D, Li T, Wang Y, Tang Y, Cui H, Tang Y, Zhang X, Chen D et al (2012) miR-132 regulates the differentiation of dopamine neurons by directly targeting Nurr1 expression. J Cell Sci. https://doi.org/10.1242/jcs.086421
156. Lungu G, Stoica G, Ambrus A (2013) MicroRNA profiling and the role of microRNA-132 in neurodegeneration using a rat model. Neurosci Lett 553:153–158
157. Yue P, Gao L, Wang X, Ding X, Teng J (2018) Ultrasound-triggered effects of the microbubbles coupled to GDNF-and Nurr1-loaded PEGylated liposomes in a rat model of Parkinson's disease. J Cell Biochem 119(6):4581–4591