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Astrocytes and Therapeutics for Parkinson's Disease

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Summary: Astrocytes play direct, active, and critical roles in mediating neuronal survival and function in various neurodegenerative disorders. This role of astrocytes is well illustrated in amyotrophic lateral sclerosis (ALS), in which the removal of glutamate from the extracellular space by astrocytes confers neuroprotection, whereas astrocytic release of soluble toxic molecules promotes neurodegeneration. In recent years, this context-dependent dual role of astrocytes has also been documented in experimental models of Parkinson's disease. The present review addresses these studies and some potential mechanisms by which astrocytes may influence the neurodegenerative processes in Parkinson's disease, and in particular examines how astrocytes confer neuroprotection either through the removal of toxic molecules from the extracellular space or through the release of trophic factors and antioxidant molecules. In contrast, under pathological conditions, astrocytes release proinflammatory cytokines and other toxic molecules that are detrimental to dopaminergic neurons. These emerging roles of astrocytes in the pathogenesis of Parkinson's disease constitute an exciting development with promising novel therapeutic targets. **Key Words:** Parkinson's disease, astrocytes, neurodegeneration, dopamine, glial–neuronal interactions, neurodegenerative diseases.

INTRODUCTION

Parkinson's disease (PD), the second most common chronic neurodegenerative disorder, is characterized primarily by the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the presence of intracellular protein aggregates known as Lewy bodies.[1,2] When the concentration of striatal dopamine is reduced below a threshold level of 70–80%, as a result of the death of the nigral dopaminergic neurons, symptoms of PD emerge.[1] The predominant motor abnormalities seen in patients with PD include bradykinesia, resting tremor, rigidity, and postural instability.

Both environmental and genetic factors play a critical role in the etiology of PD. The role for environmental toxicants has been hypothesized based on observations that parkinsonism can be caused by postencephalitic infection,[3,4] by accidental injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP),[5] and by chronic manganese intoxication.[6] Consistent with this view, human epidemiological studies have shown that rural living and exposure to herbicides, pesticides, and heavy metals increase the risk of PD.[7–11] In the last few years, exciting discoveries have been made in genetic mutations linked to early-onset and sporadic PD.[12,13] Studies of these autosomal dominant genes (the α-synuclein gene, *SNCA*; *LRRK2*, previously *PARK8*) and autosomal recessive genes (*PARK2*, alias *parkin; PARK7*, alias *DJ1; PINK1*, previously *PARK6; ATP13A2*, previously *PARK9*) have provided significant insights into mechanisms of cell death in nigral dopaminergic neurons, such as mitochondrial dysfunction, oxidative stress, neuroinflammation, and insufficient autophagic or proteasomal protein degradation.

To study the mechanisms of neurodegeneration in PD, researchers have focused their attention primarily on the affected nigral dopaminergic neurons. The neighboring glial cells, however, play a significant role in the demise of these neurons. For example, by releasing neuroinflammatory factors, activated microglia are detrimental to nigral dopaminergic neurons. This topic has been extensively reviewed elsewhere.[14,15] In this review article, we will focus our attention on recent studies highlighting astrocytes as emerging potential non-cell autonomous modulators of dopaminergic neurodegeneration. Where relevant, potential neurotherapeutic strategies targeting astrocytes will also be explored.

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ASTROCYTES AND NEURODEGENERATION

Astrocytes, the most numerous of glial cells, constitute a major class of cells in the mammalian brain and outnumber neurons by severalfold in the human brain.[16] Not only are astrocytes present in all regions of the brain, but they also appear to be organized in strategic positions that are in close contact with neuronal structures. The past stereotype of astrocytes as a kind of glue that provides mere structural support to hold the neurons in place is being increasingly revised with the recognition that these glial cells actively play critical and integral roles in mediating the physiologic and pathologic states of neurons.[17,18] The neuroprotective and neurodegenerative roles of astrocytes depend largely on the molecules that they release into and take up from the extracellular space, which is the microenvironment that astrocytes and neurons share. For example, astrocytes can release and supply neurons with neurotrophic factors such as nerve growth factor (NGF), neurotrophin-3, and basic fibroblast growth factor (bFGF), as well as metabolic substrates such as lactate and the antioxidant glutathione for the survival and proper functioning of neurons. Astrocytes also confer neuroprotection by siphoning away excess extracellular excitotoxic agents, such as glutamate, potassium, and calcium. On the other hand, when astrocytes undergo a state of gliosis in response to neuronal injury or toxic insults, together with microglia they release cytokines and chemokines that are deleterious to neurons. In the following sections, we will examine more closely recent studies on the dual role of astrocytes in neuronal survival and function in the context of PD.

ASTROCYTES CONFER NEUROPROTECTION BY RELEASING SOLUBLE MOLECULES

Glutathione

The brain is particularly vulnerable to oxidative stress, largely because of its high metabolic rate. Although through the course of evolution mitochondria have provided eukaryotic cells with a very efficient method of aerobic metabolism, an estimated 1–2% of this consumed oxygen is converted to reactive oxygen species (ROS), rather than to water.[19] This inability of the mitochondrial respiratory chain to completely reduce oxygen to water, coupled with a high rate of oxygen consumption, contributes to high ROS production by mitochondria. Under a pathological state of mitochondrial dysfunction, this rate may increase even more dramatically, as a result of leakage of electrons from the electron transport chain and the subsequent reduction of oxygen to superoxide. Consistent with this observation, oxidative stress has been proposed as a pathogenic mechanism in neurodegenerative disorders such as PD, in which mitochondrial defects have been reported.[20,21] Intrinsically, the brain is equipped with defense mechanisms against reactive oxygen species (ROS), such as the antioxidant enzymes superoxide dismutase, glutathione peroxidase, and catalase, as well as the antioxidant glutathione (GSH).[22]

Glutathione is synthesized intracellularly by γ-glutamylcysteine synthetase and GSH synthetase. The concentration of GSH in astrocytes (~ 3.8 mmol/L) is estimated to be higher than that of neurons (~2.5 mmol/L),[23,24] probably as a result of higher specific activity of the γ-glutamylcysteine synthetase in astrocytes.[25] Because of their close proximity, however, astrocytes are able to share their GSH with the neighboring neurons by releasing this antioxidant through the transporter multidrug resistance protein 1 (MRP1) into extracellular space.[26] From this shared microenvironment, GSH is cleaved by γ-glutamyltranspeptidase on astrocytic plasma membrane to generate precursors for neuronal GSH synthesis. This release appears to be a uniquely astrocytic modality; it has been demonstrated that neurons, microglia, and oligodendrocytes do not readily release GSH,[26] probably due to low levels of MRP1 in these cell types.[27] Thus, astrocytes are the major contributor to the extracellular levels of GSH. Overall, both intracellular and extracellular levels of GSH influence neuronal survival. Consistent with the neuroprotective role of GSH, reductions in GSH levels have been reported in patients and animal models of various neurodegenerative disorders. The best available data implicating deficient GSH as a pathogenic factor are for PD.[28]

The GSH content in the substantia nigra of PD patients has been observed to be significantly reduced (~40%).[29] At the cellular level, surviving nigral dopaminergic neurons display a significant loss of GSH.[30] Although the mechanism of this reduction has not been established, it is unlikely to be secondary to neuronal loss or drug treatment.[28] In genetic models of PD, astrocytes aged in cultures from *parkin*-knockout mice have lower levels of GSH than those from wild-type animals.[31] It is not clear, however, whether depletion in GSH alone may be sufficient to induce cell death, because buthionine sulfoximine, an inhibitor of γ-glutamylcysteine synthetase, does not kill dopaminergic cells.[32] It is possible that GSH depletion enhances cell death under pathological conditions.[33] Consistent with this argument, GSH depletion or its loss of function enhances dopaminergic cell death in *Drosophila* models with *parkin* mutation[34] or overexpression of α-synuclein,[35] two genetic mutations in PD.

Recognizing the critical role played by GSH in neuronal survival, attempts have been made to restore GSH levels in PD therapy. However, a recent randomized, double-blind clinical trial of parenteral GSH administration in patients with PD failed to show any clinical benefits.[36] Because the blood–brain barrier permeability to GSH is low,[37–39] this approach may not be ideal for

delivering GSH to dopaminergic neurons. An alternative strategy would be to target the molecules regulating the brain GSH system to maintain or even enhance the antioxidant capabilities of dopaminergic neurons. One such example that has gained attention recently is the NF-E2-related factor (Nrf2) transcription factor, which is known to regulate the expression of several cytoprotective genes containing the cis-acting enhancer sequence referred to as the antioxidant response element (ARE).[40] Glutathione S-transferase, which conjugates GSH to electrophilic compounds, is one of such ARE-regulated genes in astrocytes.[41] Under physiologic conditions, Nrf2 transcriptional activity is kept to a minimum by the cytosolic binding Kelch-like ECH-associated protein 1 (Keap1), which targets Nrf2 for ubiquitination and subsequent proteasomal degradation.[42] Under cellular stress, Nrf2 is stabilized and translocated to the nucleus, where it dimerizes with other transcriptional molecules and promotes the transcription of ARE-containing genes involved in glutathione, iron, and NADPH homeostasis in astrocytes.[43] In several *in vitro* and *in vivo* models, Nrf2 has been found to be preferentially induced in astrocytes.[43–45] Furthermore, Chen et al.[46] have demonstrated *in vivo* that astrocyte-confined Nrf2 overexpression is sufficient to provide neuroprotection, whereas loss of Nrf2 function enhances neuronal degeneration in a murine MPTP model of PD.

The connection of this Nrf2 system to the pathophysiology of PD has been further strengthened by the discovery that the *PARK7* gene (encoding the DJ-1 protein) is necessary for the transcriptional activity of Nrf2.[47] Homozygous mutations in *PARK7* are known to cause a recessive form of early-onset familial PD.[48,49] Although the mechanisms are incompletely understood, expression of DJ-1 confers striking protection against cellular insults. DJ-1 is expressed primarily in astrocytes in the normal human CNS and is strongly upregulated in PD.[50] Primary astroglial cultures isolated from *Park7*$^{-/-}$ mice strongly suggest that, besides promoting the expression of antioxidant genes, DJ-1 also plays an important role in the suppression of proinflammatory responses.[51] Hence, restoring or enhancing the function of the astrocytic DJ-1–Nrf2 pathway may represents a therapeutic strategy for PD patients.

Trophic factors

Astrocytes produce a variety of trophic factors that can support neuronal function. For example, basic fibroblast growth factor (bFGF or FGF-2),[52] glial cell line-derived neurotrophic factor (GDNF),[53,54] and mesencephalic astrocyte-derived neurotrophic factor (MANF)[55] have been shown to be protective in PD animal models. Among these trophic factors, GDNF has been most extensively studied and has been found to confer the most protection of dopaminergic neurons.[56] However, the success of translation of these findings to the clinic has been controversial. The side effects and conflicting results regarding effectiveness have somewhat dampened enthusiasm for these molecules.[57,58] Because these molecules are rapidly degraded and cannot permeate the blood–brain barrier, inadequate drug delivery to appropriate target neurons may contribute to these negative results. Indeed, when Salvatore et al.[59] used the same intraputamenal infusion protocol as had been used in a failed human clinical trial of GDNF, they found that GDNF levels were highest in the area surrounding the catheter and were exponentially reduced with increasing distance from the source of release, resulting in less than 10% of the putamen in the human subjects being exposed to GDNF. In the murine 6-hydroxydopamine (6-OHDA) model,[60] intrastriatal injection of MANF was found to be neuroprotective and have superior tissue distribution compared to GDNF. MANF, therefore, could be a significant therapeutic treatment for PD.

ASTROCYTES CONFER NEUROPROTECTION BY REMOVING TOXIC MOLECULES

Clearance of extracellular α-synuclein

α-Synuclein is a small, 14-kDa protein that is abundantly available in presynaptic terminals. Consistent with its cellular location, this protein has been shown to be involved in modulating synaptic vesicle function.[61] Although the exact physiological function of α-synuclein is incompletely understood, missense mutations or gene multiplication mutations leading to higher levels of wild-type synuclein cause autosomal-dominant PD.[62–65] α-Synuclein is natively unfolded in solution[66]; however, it has a propensity to form aggregates under various pathological conditions.[67] The aggregated and insoluble fibrillar form of α-synuclein constitutes a major component of the intracellular proteinaceous inclusions called Lewy bodies. Although it is still a topic of debate whether these insoluble protein aggregates are neurotoxic, neuroprotective, or incidental, the soluble oligomeric species are likely neurotoxic.

α-Synuclein has been a protein of great interest since it was discovered in the 1990s as the first genetic mutation in PD.[62–65] It has recently gained additional attention subsequent to the first post-mortem analyses in patients with PD receiving transplanted embryonic dopaminergic neurons. In three of four patients who died 11 to 16 years after transplantation, α-synuclein positive protein aggregates were present in grafted neurons; however, these aggregates were not detectable in four patients who died 4 to 9 years after the transplantation.[68–70] One possible explanation for these observations is that α-synuclein can spread from the dying host neurons to the grafted ones in a time-dependent manner.[71] Shortly after the reports of these human studies, Lee and col-

leagues demonstrated in cell culture and animal models that α-synuclein indeed is transmittable from neuron to neuron[72] and from neuron to astrocytes.[73] This unusual property has led investigators to propose that synuclein possesses "prion-like" properties.[74,75] The adverse effects of α-synuclein in neurons have been extensively studied over the years, with increased levels inversely correlating with tyrosine hydroxylase immunoreactivity in nigral dopaminergic neurons.[76] In astrocytes, however, the effects of α-synuclein remain largely unexplored.

To demonstrate the transfer of α-synuclein from neuron to astrocytes, Lee and colleagues either treated primary astrocytes with medium containing secreted α-synuclein from human neuroblastoma SH-SY5Y cells stably overexpressing this protein or co-cultured astrocytes with these stable cells.[73] In addition to detecting α-synuclein transferred to astrocytes under both conditions, the formation of large astrocytic Lewy bodies was observed in a time dependent manner. *In vivo* studies using transgenic mice overexpressing α-synuclein driven by the neuronal promoter PDGFβ yielded additional supporting evidence. In these animals, α-synuclein immunoreactivity was detected in both neurons and astrocytes. Given that astrocytes do not express α-synuclein in these animals, the detection of α-synuclein in these cells suggests it was derived from neurons and subsequently transferred intercellularly.[73]

Once inside astrocytes, α-synuclein is degraded through the lysosomal pathway.[73] Thus, astrocytes may confer neuroprotection to dopaminergic neurons by clearing excess extracellular toxic α-synuclein. A similar protective role of astrocytes has been demonstrated in models of Alzheimer's disease, where they have been shown to uptake and degrade extracellular β-amyloid protein.[77,78] Thus, strategies aimed at enhancing the removal and degradation of α-synuclein by astrocytes may be therapeutic. However, research to identify mechanisms of α-synuclein transport is still in its infancy.

ASTROCYTES INDUCE NEURODEGENERATION BY RELEASING TOXIC MOLECULES

Toxic molecules mediated by monoamine oxidase-B metabolism

The first and most direct evidence to date for the involvement of astrocytes in the loss of nigrostriatal dopaminergic is the discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced parkinsonism.[5] In 1982, a group of drug addicts in California was rushed to the emergency room presenting with a severe bradykinetic and rigid syndrome,[5] similar to that seen in PD patients. Subsequent investigations led to the discovery that, a few days earlier, these patients had self-administered MPTP-contaminated synthetic meperidine.[79] The movement abnormalities exhibited in these patients were L-DOPA responsive, as are those seen in PD patients. Since the discovery of parkinsonism caused by MPTP in these patients, this neurotoxicant has been used extensively as a model of PD in nonhuman primates, as well as in other mammalian species.[79,80] Recently, one of the surviving patients from the original 1982 cases showed a significant clinical improvement when treated with deep brain stimulation, suggesting that the MPTP model may be valid for the study of neurodegeneration in PD.[81]

The mechanism of MPTP toxicity has been studied extensively and is quite well characterized.[1] MPTP is a lipophilic prototoxin that rapidly crosses the blood–brain barrier. In astrocytes, MPTP is metabolized by monoamine oxidase-B (MAO-B) and subsequently converted to the active toxic cation of 1-methyl-4-phenylpyridinium (MPP^+). We recently demonstrated that, from astrocytes, MPP^+ is extruded into the extracellular space through the organic cation transporter 3.[82] Because MPP^+ has a high affinity for the dopamine transporter (DAT), it is taken up by the neighboring dopaminergic neurons and terminals, where it induces neurotoxicity via its inhibition of complex I of the mitochondrial electron transport chain. In addition to its affinity for DAT, MPP^+ also has high affinity for noradrenergic and serotonergic uptake transporters[83,84]; however, according to most studies, monoaminergic systems other than the nigrostriatal pathway are not prime targets for MPTP neurotoxicity. Nonetheless, direct injection of MPP^+ does produce damage to these monoaminergic nuclei.[85] Taken together, these studies suggest that perhaps the release of MPP^+ is not evenly distributed in the brain regions that are and are not affected by MPTP toxicity. Consistent with this theory, we discovered that the expression of organic cation transporter (Oct3) is selective to the group of astrocytes in the nigrostriatal regions but not in those residing in the brain regions that are not sensitive to MPTP toxicity. *In vivo* microdialysis results show that inactivation of this transporter attenuates the release of MPP^+ into extracellular space, consistent with the observation of less nigral neurodegeneration in MPTP injected $Oct3^{-/-}$ mice.[82] Thus, the selective expression of Oct3 in nigrostriatal astrocytes contributes to the selective neurodegeneration of dopaminergic neurons in this region.

Although the metabolism of MPTP to MPP^+ by MAO-B is no longer a risk for PD, it is possible that other endogenous toxic molecules maybe generated through this mechanism. For instance, the degradation of dopamine by MAO-B generates reactive dopamine quinones and other oxidative species. Tetra-isoquinoline has also been demonstrated to be metabolized by this enzyme in astrocytes to form isoquinolinium cations[86]

These lipophilic compounds, which have been linked to parkinsonism, are present in the environment, in foodstuffs, and in the brain through endogenous formation.

Primarily based on observations that MAO-B plays a critical role in MPTP toxicity and generates reactive oxidative species through dopamine metabolism, this enzyme has been a target for the treatment of PD. With aging, which is a consistent risk factor for PD, the expression levels of MAO-B also increase and its activity correlates positively with the degree of cell loss in the substantia nigra.[87–89] More direct evidence has been shown in transgenic mice with inducible overexpression of MAO-B in astrocytes under the GFAP promoter.[90] These mice were found to have age-related motor dysfunction that paralleled degeneration of neurons in the SNpc, suggesting that increased MAO-B expression and activity is sufficient to induce a parkinsonian phenotype. Conversely, smokers, who consistently have been reported to have a lower risk of developing PD, have significantly decreased MAO-B activity.[91,92] Based on such lines of evidence, the MAO-B inhibitors selegiline and rasagiline have been developed and routinely used as monotherapy or as an adjunct to L-DOPA in the treatment of PD. The efficacy of selegiline has been extensively assessed in multicenter studies, including the DATATOP trial.[93-95] MAO-B inhibitors have been found to be beneficial for ameliorating motor symptoms in PD and perhaps, although this is still controversial, offer neuroprotection as well through both the inhibition of MAO-B and independent and incompletely understood antiapoptotic effects of the propargylamine moiety common to these molecules.[96]

Proinflammatory cytokines

A review of the literature quickly reveals that the majority of findings from research on neuroinflammation points toward microglia. In fact, most, if not all, articles discussing neuroinflammation focus only on microglia. However, several lines of evidence suggest that astrocytes do release proinflammatory cytokines under pathological conditions. For instance, as already noted, a recent study shows that astrocytes are capable of removing extracellular α-synuclein released from neurons.[73] Although this uptake and subsequent degradation of α-synuclein in astrocytes may confer initial protection to neurons, once the accumulation of α-synuclein exceeds the degradation capacity of astrocytes, then aggregates of α-synuclein start to form in astrocytes. This scenario has been demonstrated when bafilomycin A1, a lysosomal inhibitor, increases the formation of detergent-insoluble α-synuclein in astrocytes.[73] Under this pathological condition, upregulation of transcripts of inflammatory cytokines such as IL-1α, IL-1β, and IL-6 and the release of TNF-α and IL-6 is detected.[73] Consistent with this toxic role of α-synuclein, the presence of α-synuclein in astrocytes correlates with nigral neuronal cell death.[97]

In addition to direct release of proinflammatory cytokines, astrocytes can also be activated by cytokines such as TNF-α and IL-1β from microglia, leading to production of reactive oxygen and nitrogen species.[98] For example, a recent study demonstrated in a coculture model that astrocytes enhance microglial inflammatory responses through an NF-κB dependent mechanism, leading to more dopaminergic toxicity.[98] The authors further showed that these effects are mediated by a novel function of Nurr1, a transcription factor crucial for the development and maintenance of dopaminergic neurons in the mesencephalon.[99–101] Notably, mutations in the *NR4A2* gene (previously *NURR1*) are associated with rare familial PD.[102–104] These mutations lead to a reduction in *NR4A2* mRNA levels in affected PD patients and cells transfected with mutant *NR4A2*.[102] Thus activating the orphan nuclear receptor NURR1 protein produces both anti-inflammatory effects and improvement in dopaminergic development and function. The development of small molecule agonists for the NURR1 protein to probe this therapeutic target are underway.[105]

Purinergic molecules

Purinergic signaling, subject of a rapidly growing field of study, is now recognized as a prevalent and complex form of intercellular communication between glia and neurons.[106] Astrocytes serve as an important source of extracellular purines. Adenosine triphosphate is released by astrocytes through mechanisms such as exocytosis, connexin or pannexin hemichannels, volume-regulated chloride channels, and $P2X_7$ receptors.[106] After release, ATP is rapidly degraded by a series of ectonucleotidases to ADP, AMP, and adenosine. In addition to this indirect mechanism, adenosine can also be released directly by astrocytes into extracellular space via equilibrative nucleoside transporters.[107] Purinergic receptors are broadly divided into two families, with one preferentially stimulated by adenosine (P1 or A receptors) and the other preferentially stimulated by ATP (P2 receptors). All adenosine receptors are G-protein coupled, whereas the P2 family is further subdivided into ionotropic (P2X) and metabotropic (P2Y) classes. In contrast to A_1, A_{2B}, and A_{3A} adenosine receptors, which have a relatively wide pattern of distribution in the CNS, the A_{2A} receptors are more restricted to the basal ganglia and are best characterized for PD[108–110]; however, P2 receptors may also be involved in neurodegeneration.[111]

GABAergic medium spiny neurons constitute the majority of neurons in the striatum, the primary input nucleus of the basal ganglia receiving dopaminergic afferents from the SNpc and glutamatergic afferents from virtually all areas of cortex. There are two anatomically and functionally distinct types of medium spiny neurons,

although they are equivalent in numbers and uniformly distributed in the striatum. Striatonigral medium spiny neurons (which represent the direct pathway) express substance P and D_1 dopamine receptors. These neurons project to the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNpr). The GPi and SNpr constitute the primary output nuclei of the basal ganglia and send inhibitory GABAergic projections to the motor nuclei of the thalamus, which in turn is excitatory to motor cortex. The net effect of this direct pathway is to facilitate motor movements by disinhibiting thalamocortical projections to motor cortex. On the other hand, the striatopallidal medium spiny neurons (which represent the indirect pathway) express enkephalin, D_2 dopamine, and A_{2A} receptors. They project to the external segment of the globus pallidus (GPe), inhibiting the subthalamic nucleus (STN), which sends excitatory glutamatergic projections to the GPi/SNpr. The net effect of this pathway is to suppress motor movements by inhibiting thalamocortical neurons. The dopaminergic input from the SNpc suppresses the indirect pathway via D_2 receptors, but stimulates the direct pathway via D_1 receptors, thus facilitating movement. Hence, any process resulting in striatal dopamine depletion results in a parkinsonian syndrome of bradykinesia, postural instability, and rigidity.

Because A_{2A} and D_2 receptors are positively and negatively coupled to adenylate cyclase, respectively,

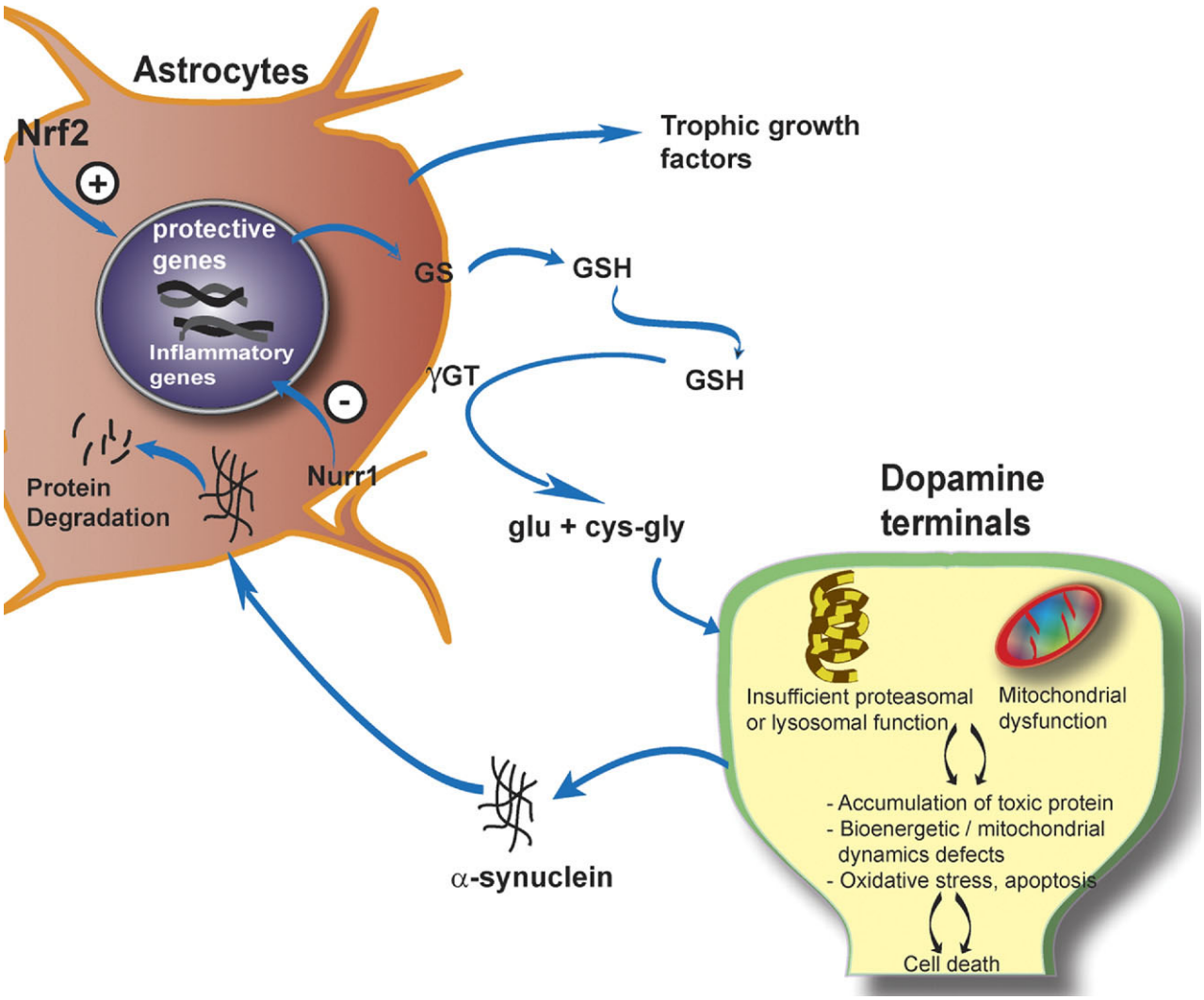

FIG. 1. Potential neuroprotective pathways of astrocytes. Genetic mutations, environmental toxicants, or a combination of both may induce nigral dopaminergic neurotoxicity through mechanisms such as mitochondrial dysfunction and insufficient degradation of misfolded proteins. Astrocytes may mediate neuroprotection through the following pathways. 1) Release of trophic growth factors, such as bFGF, GDNF, and MANF). 2) Release of glutathione (GSH), which is then cleaved by γ-glutamyltranspeptidase on astrocytic plasma membrane to generate glutamate and cysteinylglycine, which serves as precursors for neuronal GSH synthesis. 3) Activation of the transcription factor Nrf2 leads to expression of genes containing the antioxidant response element (ARE), including γ-glutamylcysteine synthetase (GS), which is involved in GSH synthesis. 4) Activation of the transcription factor Nurr1, which suppresses the production of inflammatory cytokines. 5) Removal and degradation of cytotoxic molecules, such as α-synuclein.

adenosine and dopamine exert antagonistic effects in modulating the activity of striatopallidal neurons.[110] The loss of striatal dopamine in PD leads to unopposed adenosine stimulation of this system, resulting in hyperactivity of the indirect pathway and subsequent motor dysfunction characteristic of PD. Blocking the effects of adenosine at A_{2A} therefore represents an attractive therapeutic target. Consistent with this mechanism, A_{2A} antagonists enhance D_2-mediated inhibitory effects on the striatopallidal neurons and increase D_1-mediated stimulatory effects on the striatonigral neurons, resulting in alleviation of parkinsonian motor symptoms in rodents[112] and MPTP-treated nonhuman primates,[113] as well as in humans.[114–116]

Together these studies strongly support the role of A_{2A} antagonists as novel nondopaminergic therapy in relieving parkinsonian motor deficits. The beneficial effects of blocking A_{2A} may even extend to protecting dopaminergic neurons from degeneration. For example, epidemiological studies consistently report that intake of caffeine (a nonselective adenosine receptor antagonist), whether from coffee or noncoffee sources, reduces risk of developing PD.[117,118] More direct evidence is provided in a mouse model of PD, in which caffeine or genetic deletion of A_{2A} attenuated MPTP- and 6-OHDA-induced dopaminergic neurotoxicity.[119-121] The mechanism of this neuroprotection has been attributed to the attenuation of neuroinflammation induced by astrocytes and

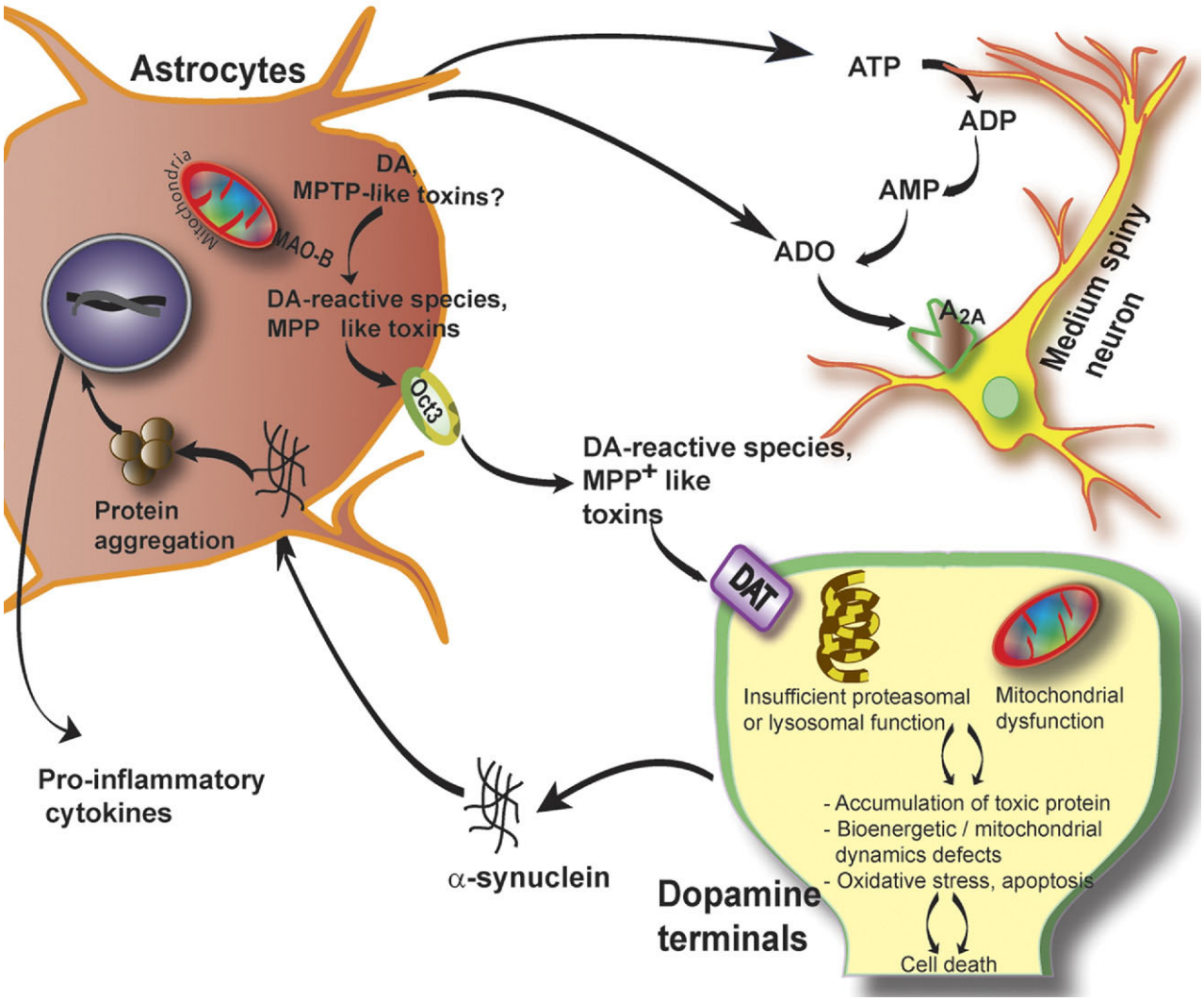

FIG. 2. Potential neurotoxic pathways of astrocytes. Astrocytes may also adversely affect the survival and function of dopaminergic neurons through the following mechanisms: 1) Release of proinflammatory cytokines under pathological conditions such as accumulation of aggregated α-synuclein. 2) Monoamine oxidase-B (MAO-B) mediated release of cytotoxic molecules such as dopamine-related oxidants and MPP^+-like organic cations through the organic cation transporter (Oct3) into the extracellular space where they are subsequently transported into DA neurons through the dopamine transporter (DAT). 3) Astrocytes can also release adenosine (ADO) directly or indirectly via ATP. ADO may increase movement disorders in patients with PD through the A_{2A} receptors in striatal medium spiny neurons.

microglia, as well as the reduced glutamate release from the presynaptic glutamatergic terminals or astrocytes in the striatum.[114–116] However, additional studies are needed to elucidate the neuroprotective mechanism of A_{2A} inhibition.

Another relatively unexplored aspect of this system is the cellular and molecular source of extracellular adenosine. Astrocytes remain a likely candidate as the cellular source, by virtue of their well-studied mechanisms of purine release in other brain regions and in disease models.[122,123] Regardless of the cellular source, the mechanism of release (i.e., exocytotic or transmembrane protein channels) and whether it is adenosine or another precursor molecule (ATP, cAMP) that is released into the extracellular compartment, further investigation is warranted, because they constitute additional potential therapeutic targets.

CONCLUSION

Since the recognition of PD more than 200 years ago, the primary focus of research in elucidating the mechanisms of cell death in this neurological disorder has been the affected dopaminergic neurons. However, as reviewed in this article and summarized in FIGS. 1 and 2, through diverse pathways of release or removal of protective and neurotoxic molecules, a picture starts to emerge that astrocytes, either directly or indirectly, play a role in survival and function of dopaminergic neurons in experimental models of PD.

One challenge in the study of how astrocytes modulate neurotoxicity in PD is the lack of suitable models of neurodegeneration in this disorder. Despite their popularity, neurotoxins such as MPTP and 6-OHDA still remain acute toxic models, which, understandably, may not accurately model PD pathogenesis. With recent discoveries of genetic mutations in PD, various animal models have been developed; however, these genetic models are not suitable for the study of neurodegeneration.[124] Animal models expressing these genetic mutations in astrocytes are currently lacking, but if developed might also be useful tools to further investigate the role of astrocytes in pathogenesis and therapeutic development for PD. Despite these obstacles, current evidence supports the idea that astrocytes are fully capable of modulating the survival and function of dopaminergic neurons. Therapeutic strategies targeting astrocytes for PD treatment therefore warrant consideration.

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