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Parkinson's disease: changes in apoptosis-related factors suggesting possible gene therapy

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Summary. Specific degeneration of the nigrostriatal dopamine (DA) neurons of the substantia nigra pars compacta and the resulting loss of nerve terminals accompanied by DA deficiency in the striatum are responsible for most of the movement disturbances called parkinsonism, i.e., muscle rigidity, akinesia, and resting tremor, observed in Parkinson's disease (PD). We and other workers have found changes in the levels of cytokines, neurotrophins, and other apoptosis-related factors in the nigro-striatal region of postmortem brain and/or in the cerebrospinal fluid (CSF) from PD patients, or from animal models of PD such as 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP)-induced PD in mice or 6-hydroxydopamine (6-OHDA)-induced PD in rats. The most remarkable changes observed specifically in the nigrostriatal region were decreased levels of neurotrophins supporting DA neurons. These results indicate that the process of cell death in the nigrostriatal DA neurons in PD may be the so-called programmed cell death, i.e., apoptosis. Thus gene therapy for PD should aim both at supplementing the decreased striatal DA level by introducing the genes of DA-synthesizing enzymes into non-DA cells in the striatum and at supporting or restoring DA neurons by preventing apoptosis by introducing genes that block the process of apoptosis.

Keywords: Parkinson's disease, cytokines, neurotrophins, apoptosis, gene therapy.

Abbreviations

AADC aromatic L-amino acid decarboxylase, *AD* Alzheimer's disease, *BDNF* brain-derived neurotrophic factor, *bFGF* basic fibroblast growth factor, *BH4* (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin, *CSF* cerebrospinal fluid, *DA* dopamine, *ELISA* enzyme-linked immunosorbent assay, *GCH* GTP cyclohydrolase I, *GDNF* glial cell line-derived neurotrophic factor, *EGF* epidermal growth factor, *IL* interleukin, *MAO-B* monoamine oxidase type B, *MHC-I* class I major histocompatibility complex, *MPP+* 1-methyl-4-phenyl-

pyridinium ion, *MPTP* 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *NH2P3* D-erythro-7,8-dihydroneopterin triphosphate, *NGF* nerve growth factor, *6-OHDA* 6-hydroxydopamine, *PD* Parkinson's disease, *ROS* reactive oxygen species, *sFas* soluble Fas, *TH* tyrosine hydroxylase, *TNF* tumor necrosis factor, *TNFR1* tumor necrosis factor receptor 1, *TGF* transforming growth factor.

Introduction

Parkinson's disease (PD) is characterized by deficiency of neurotransmitter dopamine (DA) at the nerve terminals of nigrostriatal DA neurons in the striatum caused by selective neurodegeneration and the resultant loss of the DA neurons in the substantia nigra pars compacta. The characteristic clinical symptom of PD is a movement disorder called parkinsonism, i.e., rigidity, bradykinesia, and resting tremor. Familial PD constitutes a small percentage of PD cases (approximately 5%), and the most of PD is sporadic and aging-related. PD is the second most common neurodegenerative disease after Alzheimer's disease (AD).

The pathogenesis of sporadic PD is still enigmatic (Foley and Riederer, 1999): many factors are speculated to operate in the mechanism of cell death of the nigrostriatal DA neurons in PD, e.g., oxidative stress and cytotoxicity of reactive oxygen species (ROS), disturbance of intracellular calcium homeostasis, endogenous or exogenous neurotoxins (Nagatsu, 1997, 2002), and mitochondrial dysfunction, especially of complex I of the electrotransport system.

Recent studies have been focused on immune responses and the factors of the pathways of programmed cell death, i.e., apoptosis, that might be involved in the neurodegeneration in PD (Anglade et al., 1997; Hirsch et al., 1999; Jellinger, 2000; Mogi and Nagatsu, 1999c; Nagatsu and Mogi, 1998; Nagatsu et al., 1999a, 2000a,b; Wullner et al., 1999).

We measured biochemically changes in the concentrations of cytokines and neurotrophins in postmortem brain tissues from PD patients by enzyme-linked immunosorbent assay (ELISA). We have obtained, specifically from the nigrostriatal DA region of the postmortem brains of patients with PD as well as from that of animal models of PD, the following evidences suggestively implicating the immune response and apoptosis in PD: (1) a progressive increase in the neopterin/biopterin ratio in the cerebrospinal fluid (CSF) from patients with PD; (2) increased levels of β2-microglobulin, the light chain of the major histocompatibility complex class I (MHC-I); (3) increased levels of proapoptotic cytokines; (4) decreased levels of antiapoptotic neurotrophins, (5) increased levels of apoptosis-related proteins or apoptosis-producing caspases (Table 1). These results suggest that the nigrostriatal DA cell death in PD may be caused by a glial immune response and subsequent occurrence of apoptosis. Although the concept of programmed cell death (apoptosis) in PD is still controversial in itself, these data from postmortem brains of PD and animal models of parkinsonism strongly indicate the presence of a proapoptotic environment in the nigrostriatal DA region in PD.

Table 1. Changes in cytokines, neurotrophins, and apoptosis-related proteins in the brain and/or ventricular or lumbar cerebrospinal fluid (CSF) in Parkinson's disease

Brain (striatum/substantia nigra)		CSF	
		Ventricular CSF	Lumbar CSF
BDNF	↓↓		
NGF	↓↓		
GDNF	→		
IL-1β	↑	↑	
TNF-α	↑↑		↑↑
IL-2	↑	↑	ND
IL-4		↑	ND
IL-6	↑	↑	↑
EGF	↑		
TGF-α	↑	↑	ND
TGF-β1	↑	↑	ND
TGF-β2		↑	
b-FGF	→		
β2-microglobulin	↑	↓	↓
Bcl-2	↑	ND	ND
sFAS	↑	ND	ND
TNF R1(p 55)	↑		
cas pase 1	↑		
cas pase 3	↑		

ND not detectable

The neuronal cell death in PD is a slow-going process, but recently proposed "one-hit model of cell death" in PD may fit the concept of apoptosis (Clarke et al., 2000).

The apoptosis theory in PD would contribute to the development of the ongoing gene therapy of PD. Supplementation of deficient DA in the striatum by introducing DA-synthesizing enzymes has been the main approach in the gene therapy for PD, but the introduction of antiapoptotic genes would be expected to contribute to neuroprotective and/or neurorestorative gene therapy for PD.

Immune response in the brain in Parkinson's disease

The brain is generally considered to be a "privileged" site, i.e., one free from immune reactions, since it is protected behind the blood-brain barrier. However, recent findings revealed that immune responses may occur in the brain, probably by microglia activation that produces proinflammatory cytokines (Benveniste, 1992; Sei et al., 1995). We found suggestive evidences of immune responses, such as a significant increase in the neopterin/biopterin ratio in the CSF during the progression of PD (Fujishiro et al., 1990), and an increased level of β2-microglobulin [the light chain of class I major histocompatibility complex (MHC-I)] in the striatum in PD (Mogi et al., 1995b). Neopterin

is a metabolite of D-erythro-6,7-dihydroneopterin triphosphate (NH2P3). NH2P3 is synthesized in DA, noradrenaline, adrenaline, serotonin, and NO neurons as an intermediate of (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4), which is a cofactor of tyrosine hydroxylase (TH), the enzyme catalyzing the first step of DA biosynthesis (Nagatsu et al., 1964), and also in NH2P3-producing activated microglia or astrocytes (Nagatsu and Ichinose, 1999; Nagatsu et al., 1999b). Thus neopterin is produced via NH2P3 derived either from DA neurons or from activated glial cells. During the progression of PD, the production of biopterin as well as that of neopterin derived from nigrostriatal DA neurons is greatly decreased; but neopterin formation from activated glial cells may be increased, resulting in a significantly increased neopterin/biopterin ratio. Increases in the neopterin/biopterin ratio during the progression of PD suggest activation of microglia. Activation and production of neopterin in the PD brain is thought to occur specifically in the nigrostriatal region, since GTP cyclohydrolase I activity, the first and rate-limiting enzyme for BH4 synthesis, was found to be decreased specifically in that region (Nagatsu et al., 1986), in contrast to the increased level of β2-microglobulin, the light chain of MHC-I (Mogi et al., 1995b).

McGeer et al. (1988, 1995) also proposed that the inflammatory response system of the brain plays a role in PD based on the finding of reactive microglia bearing MHC-II antigen in the substantia nigra in PD.

Changes in cytokines and neurotrophins in the brain in Parkinson's disease

Since the increased neopterin/biopterin ratio in the CSF and the increased level of β2-microglobulin in the striatum in PD suggest that such increases may be due to their increased production of neopterin and β2-microglobulin from the glial cells in the nigrostriatal region owing to immune activation, we further measured various cytokines, which are related to immune response and apoptosis, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-2, IL-4, IL-6, epidermal growth factor (EGF), transforming growth factor (TGF)-α, TGF-β1, and basic fibloblast growth factor (bFGF) in the brain (striatum) and/or in the ventricular and lumbar CSF in PD (Mogi et al., 1994a,b, 1995a,b, 1996a,b,c). Among these cytokines, the levels of proinflammatory cytokines such as TNF-α, IL-1β, or IL-6 were found to be markedly increased, specifically in the nigrostriatal region in PD.

We also found that the level of TNF-α receptor R1 (TNFR1, p53) was elevated in the substantia nigra in PD in comparison with that of controls (Mogi et al., 2000a). In agreement with our results by enzyme-linked immunosorbent assay (ELISA), Boka et al. (1994) found TNF-α immunoreactive glial cells in the substantia nigra in PD, and other workers also reported increased cytokine levels in PD de novo without L-DOPA treatment: IL-1β and IL-6 in lumbar CSF (Blum-Degan et al., 1995) and TGF-β1 and TGF-β2 in ventricular CSF (Vawter et al., 1996).

TNF-α was found to bring about the apoptotic death of NGF-deprived sympathetic and sensory neurons. Function-blocking antibodies against either TNF-α or TNFR1 rescued many sympathetic and sensory neurons following

NGF deprivation in vitro (Barker et al., 2001). A similar situation may exist in the nigrostriatal region in PD.

Neurotrophins [nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4/5] are proteins that support the differentiation and survival of neurons, and act as antiapoptotic factors for neurons. In contrast to increased levels of cytokines, the level of BDNF in the nigrostriatal DA region (on the order of ng/mg protein) was significantly lower in PD than in controls. The level of NGF (on the order of pg/mg protein) was also significantly decreased in the substantia nigra in PD in comparison with that in controls (Mogi et al., 1999b,c).

Depletion of neurotrophins such as BDNF and NGF triggers the process of apoptosis, as these neurotrophins are known to be neuroprotective for DA neurons. Thus, the lack of neurotrophins may be involved in the pathogenesis of PD during the progressive neurodegeneration of the nigrostriatal DA neurons. Glial cell line-derived neurotrophic factor (GDNF) is a neurotrophin that has attracted considerable interest in relation to PD, because of its particularly strong effects on DA neurons (Akerud et al., 2001; Gash et al., 1996; Kordower, 2000). We showed that GDNF levels (on the order of pg/mg protein) were significantly higher in the nigrostriatal DA regions (substantia nigra, caudate nucleus, putamen) than in the cerebellum and frontal cortex from either control or PD brains. Interestingly, the content of GDNF showed no significant difference in the nigrostriatal region between PD and control patients. This is in contrast to the markedly reduced levels of BDNF or NGF specifically in that region in PD. The unchanged level of GDNF in PD could be due to compensatory production in glial cells, which occurs with neither BDNF nor NGF (Mogi et al., 2001). Interestingly, the concentration of another DA neuron-protective growth factor, basic FGF (bFGF), which was shown to be abundant in the nigrostriatal region (on the order of ng/mg protein), was unchanged between control and PD striatum (Mogi et al., 1996c), also suggesting its compensatory production in glial cells.

Changes in apoptosis-related factors in the nigrostriatal region in Parkinson's disease

Deprevation of neurotrophins and the presence of proinflammatory cytokine TNFα can be a potent apoptotic signal (Raff, 1992). So we have examined changes in the levels of apoptosis-related factors in PD brains. The Fas antigen/APO-1/CD95 is a cell-surface receptor protein known to trigger apoptosis upon binding the Fas ligand, and belongs to the TNF-α/NGF receptor family. Fas antigen and two TNF receptors, p55 and p75, were implicated in triggering cell death upon stimulation by natural ligands, i.e., TNF-α and Fas ligands (Nagata and Goldstein, 1995).

Molecular cloning and nucleotide sequence analysis revealed a human Fas mRNA variant capable of encoding a soluble Fas (sFas) molecule lacking the transmembrane domain because of the deletion of an exon encoding this region. sFas protects against Fas-mediated apoptosis. We found that the con-

centrations of sFas in nigrostriatal DA regions were significantly higher in PD than in controls (Mogi et al., 1996d).

We also found that the concentration of antiapoptotic bcl-2 protein, which is localized in several cell components such as inner and outer mitochondrial membranes (Akao et al., 1994) in cells in the nigrostriatal DA region, was significantly higher in PD patients than those in controls (Mogi et al., 1996b). The antiapoptotic activity of bcl-2 is linked to reduced generation of ROS (Hockenbery et al., 1993; Kane et al., 1993). Because the upregulations of sFas and bcl-2 were seen neither in control tissues nor in PD cerebral cortex, these elevations are topographically specific in PD.

Members of a novel family of aspartate-specific cysteine proteases, which include caspase1 (IL-1β-converting enzyme) and caspase 3, have been implicated as mediators of apoptotic cell death (Cerretti et al., 1992; Kumar, 1995). Both caspase-1- and caspase-3-like proteases are involved in TNF and Fas-receptor-mediated apoptosis (Nagata, 1997). The activities of caspases 1 and 3 were significantly higher in the substantia nigra from PD patients than in that in the brain from control patients (Mogi et al., 2000a). Activated caspase 3 was also detected immunohistochemically and was proposed to be the final effector in the apoptotic cell death of DA neurons in PD (Hartmann, 2000). Since both caspases 1 and 3 and TNFR1 may play important roles in apoptotic cell death through the TNF-α-induced signaling pathway, the presence of a proapoptotic environment in the nigrostriatal region of the PD brain suggests vulnerability of neurons and glial cells towards a variety of noxious factors. Ribozyme-mediated inhibition of caspase-3 activity reduced apoptosis induced by 6-hydroxydopamine (6-OHDA) in PC12 cells (Xu et al., 2001).

In another human PD brain study, a significantly higher percentage of DA neurons in the substantia nigra pars compacta displayed caspase-8 activation as indicated by immunoreactivity (Hartmann et al., 2001b). Caspase-8 may cause the release of cytochrome c from mitochondria to trigger caspase-3 activation.

Cytokine and neurotrophin changes in the brain in PD animal models

There are several animal models of PD (Beal, 2001). Changes in cytokines and neurotrophins similar to those seen in the brain in PD were observed in the nigrostriatal region of PD animal models. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces apoptotic cell death of the nigrostriatal DA neurons (Tatton and Kish, 1997). In PD mice produced by repeated intraperitoneal injection of MPTP, the IL-1β concentration was increased 23-fold and the NGF concentration was decreased to about 50% of normal, specifically in the striatum (Mogi et al., 1998). These results of the increased IL-1β and decreased NGF levels specifically in the striatum of MPTP-treated PD mice agree with the data from postmortem PD brains.

In hemiparkinsonian rats produced by injecting 6-OHDA into one side of the ventrotegmental bundle without or with L-DOPA treatment, the levels of TNF-α were significantly increased only in the substantia nigra and striatum of the injected side. L-DOPA administration did not produce any significant

changes in TNF-α levels in 6-OHDA-treated or control sides of any of the brains (Mogi et al., 1999a). These results agree with the changes in the TNF-α levels in the striatum and lumbar CSF in PD patients, and also suggest that the increased cytokine levels in PD patients may not be due to the secondary effects of L-DOPA therapy. The increase in the level of TNF-α in rats with a 6-OHDA-lesioned striatum was suppressed by immunophilin ligand FK506 (Mogi et al., 2000b). Immunosuppressant FK506 may prevent activation of microglial cells by 6-OHDA injection, resulting in a decrease in TNF-α induction in the nigrostriatal DA region of the rats. Apoptosis induced by an endogenous MPTP-like neurotoxin, N-methyl (R) salsolinol (Naoi et al., 2000), was found to be mediated by activation of caspase 3 (Akao et al., 1999).

MPTP-like and PD-producing endogenous neurotoxins and apoptosis

The cell death caused by MPTP is thought to be apoptotic (Tatton and Kish, 1997). MPTP is a synthetic N-methylated amine product, easily enters the brain from the blood stream through the blood-brain barrier, and is oxidized to the 1-methyl-4-phenylpyridinium ion (MPP+) by monoamine oxidase type B (MAO-B) in glial cells. Then MPP+ is transported into DA neurons via DA transporter, and kills the DA neurons by inhibiting complex I of mitochondria and producing ROS. Various MPTP-like endogenous neurotoxins have been found in the PD brain, such as isoquinolines and β-carbolines (Nagatsu, 1997, 2002). R-N-methyl-salsolinol (R-N-methyl-6,7-dihydroxytetrahydroisoquinoline), which may be synthesized from DA in the DA neurons, was reported to inhibit complex I in mitochondria, thus producing ROS, and to cause apoptotic cell death, as evidenced by DNA fragmentation (Naoi et al., 1996). Like MPTP, β-carbolines were also found to destroy the nigrostriatal DA neurons after their direct injection into the striatum (Matsubara et al., 1995). Endogenous isoquinolines and β-carbolines in the brain, like MPTP, are assumed to be first N-methylated and then oxidized by MAO to the corresponding isoquinolinium ions or β-carbolinium ions. The mechanism of the DA cell death by PD symptom-producing neurotoxins, MPTP/MPP+, isoquinoline/isoquinolinium ion, and β-carboline/ β-carbolinium ion, may be similar and apoptotic. It remains to be examined whether the isoquinoline/isoquinolinium ion or β–carboline/β-carbolinium ion produces changes in cytokines or neurotrophins in the nigrostriatum similar to those effected by MPTP/MPP+.

The future prospects of apoptosis study in PD

All data showing the increased levels of proapoptotic cytokines, the decreased levels of antiapoptotic neurotrophins, and the increased levels of apoptosis-related factors such as caspases may indicate the apoptotic cell death of the nigrostriatal DA neurons. However, there still remain several questions to be asked about the roles of cytokines and neurotrophins in relation to the apoptotic death of nigrostriatal DA neurons.

The first question is the specificity of the observations both in the nigrostriatal region and in the disease PD. Human postmortem brain studies

using punched-out tissues from various brain regions can give valuable information directly on the biochemical changes in the nigrostriatal region in PD, but mostly at the advanced stage of the disease. Postmortem degradation of proteins in postmortem tissues must be considered. Samples contain both glial cells and neurons. Therefore, caution is necessary on postmortem brain studies, because without appropriate controls in terms of postmortem time and the cause of death the significance of the observation is limited. In our initial studies on cytokines and neurotrophins, we used brain tissue samples from the brain bank at the University of Würzburg, Germany (Dr. Riederer P), and later studies were carried out on samples from the brain banks in Japan (Dr. Mizuno Y at Juntendo University, Dr. Kuno S at Utano National Hospital). The data from specimens obtained from these 2 brain banks seem to be in agreement. Furthermore, the results from in PD-model animals such as MPTP-PD mice and 6-OHDA-PD rats, without such problems that exist in human postmortem brain studies, were similar to those from PD patients. Thus the results on human brain biochemistry are thought to be specific both in the nigrostriatal region and in PD.

Second, the origin of cytokines and neurotrophins and their interactions are other important questions. Immune-activated glial cells, i.e., microglia and astrocytes, may produce cytokines and neurotrophins, but neurons may also do so (Aloisi, 2001). Thus the interrelation and the sequence of the observed changes between glial cells and DA neurons must be clarified. Data using immunohistochemistry should be compared with the biochemical data. TNF-α immunoreactive glial cells were found in the substantia nigra (Boka et al., 1994). Activated forms of caspase 3 (Hartmann et al., 2000) and caspase 8 (Hartmann et al., 2001b) were proved by immunohistochemistry. The Bcl-2 protein level was increased in the nigrostriatal region in PD (Mogi et al., 1996), but the numbers of bcl-2-postive neurons and Bax (a proapoptotic bcl-2 member)-positive neurons were found to be unchanged and those of antiapoptotic bcl-XL-positive neurons specifically decreased, as judged by immunohistochemistry (Sugita Y, Goto K, Mori H, Hatano T, Kato E, Ohta S, Mizuno Y, personal communication). This discrepancy suggests that increased bcl-2 concentrations may be due to overproduction from glial cells as a compensatory mechanism, as described below. In vitro data obtained by immunohistochemistry also indicate that Bax may not play a central role in the apoptotic death of DA neurons (Hartmann et al., 2001a). Thus bcl-XL may be an important factor in the death of DA neurons in PD. The increases in cytokines in the nigrostriatal DA region suggest the induction of apoptotic cell death of DA neurons, but glial cells may die first by apotosis, and then the DA neurons.

Third, the increased levels of cytokines and the decreased levels of neurotrophins may cause apoptotic cell death, but these changes could be secondary or compensatory responses. Cytokines including TNF-α mediate the cell death of a sensitive population of DA neurons, and could be a causative agent in PD (McGuire et al., 2001). IL-1β is a proinflammatory cytokine but pleiotropic, and either promotes inflammation and signals leading to cell death or exerts neuroprotective effects (Mason et al., 2001). As

described above, the increased levels of antiapoptotic protein bcl-2 could be a compensatory response. The unaltered levels of GDNF and bFGF, which specifically protect DA neurons, in the nigrostriatal region in the PD brain compared with the control one could also be compensatory reactions against the decreases in neurotrophins such as BDNF or NGF, in which compensation may not occur. bFGF was reported to be lost in substantia nigra neurons in PD (Tooyama et al., 1993). Thus the unaltered concentration in that region may be due to compensatory production from glial cells.

Fourth, the signaling pathway to apoptotic neuronal death in the nigrostriatum in PD remains to be clarified. TNF-α induces trimerization of TNFR1 upon binding. TNF receptor-associated death domain (TRADD) and Fas-associated death domain (FADD) activate caspase 8 (Schulze-Osthoff et al., 1998). Caspase 8 may cause activation of caspase 3 either directly or via cytochrome c release from mitochondria (Hartmann et al., 2001b). Neurotrophins regulate neuronal apotposis through the action of critical protein kinase cascades, such as the phosphoinositide 3-kinase/Akt and mitogen-activated protein kinase pathways (Yuan and Yankner, 2000).

MPTP activates c-Jun NH(2)-terminal kinase (JNK) and the upstream regulatory kinase MKK4 in nigrostriatal neurons in vivo, and JNK may also be activated in PD. An in vitro study using PC12 cells indicated that NGF prevented MPTP-induced cell death via the Akt (protein kinase B, PKB) pathway by suppressing caspase-3-like activity (Shimoke and Chiba, 2001). JNK plays a key role in apoptotic cell death. In some neurons JNK is thought to initiate cell death by the activation of c-Jun. JNK activation is a key event in apoptotic death induced by NGF withdrawal, where its point of action lies upstream of mitochondrial dysfunction in sympathetic neurons (Harding et al., 2001).

The recent discovery of the causative genes of familial PD, i.e., α-synuclein for autosomal dominant PD (Polymeropoulos et al., 1997) and parkin for autosomal recessive juvenile PD (Kitada et al., 1998), may give important clues for elucidating the signaling pathway of apoptotic neuronal death in sporadic PD. Parkin protein was identified as a ubiquitin-protein ligase (E3, Shimura et al., 2000). Pael (*p*arkin-*a*ssociated *e*ndothelin receptor-*l*ike) receptor (Imai et al., 2001) and a newly recognized 22-kDa glycosylated form of α-synuclein (αSp22, Shimura et al., 2001) were identified as substrates of parkin. Thus parkin is linked to the ubiquitin pathway, and its function should shed new light on the mechanism of sporadic PD (Tanaka et al., 2001; McNaught et al., 2001). Pael receptor is a putative G protein-coupled integral membrane polypeptide, the accumulation of which can lead to endothelial reticulum (ER) stress. Since the expression level of the Pael receptor in neurons of the substantia nigra is significantly high, ER stress might contribute to the degreneration of the substantia nigra DA neurons. Pathological accumulation of the newly recognized form of α-synuclein may also accelerate neuronal loss and cause younger age of disease onset. In either case, the accumulation of denatured proteins by the loss of function of E3 ubiquitin-protein ligase may be linked to neuronal death. Aberration in the cellular protein degradation pathway may be a common process in neurodegenerative

disorders such as PD and AD (Krüger et al., 2000). In sporadic PD, mitochondrial dysfunction may produce ROS formation and ATP depletion (Mizuno et al., 1998). Moderate ATP decrease may cause apoptotic cell death; but severe ATP depletion, necrosis (Akao et al., 1994). Chronic partial inhibition of mitochondrial complex I with oxidative damage without ATP depletion by the lipophilic pesticide rotenone in rats was found to produce the anatomical, neurochemical, behavioral, and neuropathological features of PD with cytoplasmic inclusions reminiscent of Lewy bodies (Betarbet et al., 2000). α-Synuclein and ubiquitin are components of Lewy bodies. These facts may indicate a close link between familial PD and sporadic PD. The accumulation of abnormal protein in the nigrostriatal DA neurons in familial PD may also be linked to the apoptotic pathways that may also be activated by oxidative stress and mitochondrial dysfunction in sporadic PD. Thus, the causes of PD might be multiple but link ultimately to the apoptotic pathways.

Gene therapy of PD based on the changes in apoptosis-related factors

Gene therapy for PD has been focused on the supplementation of DA in the striatum by introducing the genes for DA-synthesizing enzymes such as tyrosine hydroxylase (TH), aromatic L-amino acid decarboxylase (AADC), and GTP cyclohydrolase I (GCH), the latter of which synthesizes the cofactor BH4 for TH (e.g., Shen et al., 2000). We previously reported that the synthesizing capacity of TH is highly enhanced in the residual DA neurons in PD although overall activity of the enzyme is decreased simply due to the fact that TH is reducing from the degenerating neurons (Mogi et al., 1988). This means that gene therapy for TH supplementation in the nigrostriatal DA neurons may not be appropriate. The observed efficacy of gene therapy targeting at the striatum may be due to supplementation of TH in other neurons than in residual DA neurons (Shen et al., 2000). The genes of antiapoptotic neurotrophins and apoptosis-related factors could be used for the neuroprotective or neurorestorative gene therapy of PD, e.g., GDNF (Bjorklund et al., 2000; Kordower et al., 2000; Connor et al., 2001) or Apaf-1 dominant negative inhibitor (Mochizuki et al., 2001). Drugs that produce antiapoptotic effects could be effective for preventing or blocking the cell death of DA neurons; DA2 receptor (D2R) agonists such as apomorphine (Ohta et al., 2000), deprenyl (an MAO-B inhibitor), and deprenyl-related propargylamines have neuroprotective effects and increase the levels of BDNF, NGF, and GDNF. Gene therapy would be able to produce neurotrophins such as BDNF, NGF, or GDNF or to block the apoptosis pathway for a long period to protect against disease progression as a neuroprotective therapy (Olson, 2000; Xia et al., 2001). ROS could also be the target of multi-neuroprotective strategies in the gene therapy of sporadic PD as shown in MPTP- and 6-OHDA-PD models (Grunblatt et al., 2000).

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