Mitochondrial Dysfunction and Biogenesis in the Pathogenesis of Parkinson's Disease

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Parkinson's disease (PD) is a progressive neurological disorder marked by nigrostriatal dopaminergic degeneration and development of cytoplasmic aggregates known as Lewy bodies. The impact of this disease is indicated by the fact that mortality is two to five times as high among affected persons as among age-matched controls. However, the cause of PD is still unknown and no cure is available at present. Several biochemical abnormalities have been described in the brains of patients with PD, including oxidative stress and mitochondrial dysfunction. Recent identification of specific gene mutations that cause PD has further reinforced the relevance of oxidative stress and mitochondrial dysfunction in the familial and sporadic forms of the disease. The proteins that are reported to be related to familial PD–PTEN-induced putative kinase 1 (PINK1), DJ-1, α-synuclein, leucine-rich repeat kinase 2 (LRRK2), and, possibly, parkin–are either mitochondrial proteins or are associated with mitochondria, and all are involved in pathways that elicit oxidative stress or free radical damage. Mitochondria are continually exposed to reactive oxygen species and accumulate oxidative damage more rapidly than the rest of the cell. Therefore, Parkinson's disease has been suggested to be associated with mitochondrial dysfunction. Since mitochondria are the major intracellular organelles that regulate both cell survival and death, clarifying the involvement of mitochondrial dysfunction and biogenesis during the process of PD could provide treatment strategies that might successfully intervene in the pathogenesis and slow the progression of the disease. *(Chang Gung Med J 2009;32:589-99)*

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disease, affecting 1 to 2% of the population over the age of 65.[1] PD is a chronic, progressive disease caused by relentless degener-

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ation of specific neuronal populations in the brain, notably the dopaminergic neurons of the substantia nigra pas compacta, and age is the single most consistent risk factor in the disease.[2,3] With the increasing age of the general population, the prevalence of PD will rise steadily.[4] The impact of this disease is indicated by the fact that mortality is two to five times as high among affected persons as among age-matched controls, resulting in a marked reduction in life expectancy, as well as debility during life.[5] However, the cause of PD is still vague and no cure is available at present for this disease.[5,6] In the past decade, there have been many major advances in identifying discrete genetic and molecular causes of parkinsonism and mapping the events involved in nigral cell death. There is increasing evidence that mitochondrial dysfunction may be linked to neurodegenerative diseases through different pathways, including free-radical generation, deficiency in the mitochondrial respiratory enzyme complex and mitochondrial permeability transition.[1,7-13] This can eventually lead to both the apoptotic and necrotic death of neurons. Recently, several specific gene mutations that cause familial PD have been identified, including phosphatase and tension homologue (PTEN)-induced putative kinase 1 (PINK1), DJ-1, α-synuclein, leucine-rich repeat kinase 2 (LRRK2) and parkin. The finding that these proteins are either mitochondrial proteins or are associated with mitochondrial dependent cell death has further reinforced the relevance of oxidative stress and mitochondrial dysfunction in the development of the disease.[14,15] Therefore, clarifying the involvement of mitochondrial dysfunction and biogenesis during the pathogenesis of PD could be helpful in better understanding the pathogenesis of this human disease and the development of therapeutic approaches. Below, we briefly summarize several important factors concerning this cellular organelle including the biology, genetics, oxidative stress elicited by mitochondrial dysfunction, and role of mitochondria in the process of cell death, and then focus on the crucial role of mitochondrial functions, especially complex I activity, in maintaining dopaminergic neuronal integrity. We further discuss recent advances in the genetics of PD and the importance of these genes in maintaining mitochondrial functions. Finally, a hypothesis of the involvement of mitochondrial biogenesis induced by increased oxidative stress and life or death decision making during the process of neuronal damage are discussed.

Mitochondrial biology, genetics and oxidative stress

Mitochondria are organelles enclosed by a double membrane and are essential for cell viability. Mitochondria have two well-defined compartments, the matrix, surrounded by the inner membrane (IM), and the intermembrane space, surrounded by the outer membrane (OM).[16,17] The IM contains the protein complexes necessary for the electron transport chain, the F_0F_1-ATPase and the adenine nucleotide translocator (ANT). In order to create a large surface area for ATP production, the IM is folded into numerous cristae.[17,18] These cellular organelles produce most of the cell's energy in the form of ATP by oxidative phosphorylation (OXPHOS).[19] While most of the proteins in mitochondria are encoded by nuclear DNA and imported into the organelles, 13 are encoded by mitochondrial DNA (mtDNA). Human mtDNA is a 16,569-bp circular double-stranded DNA molecule coding for 13 polypeptide components of the mitochondrial OXPHOS machinery, 2 ribosomal RNA molecules and a set of 22 transfer RNA molecules.[20-23] More than 99% of mitochondrial proteins are encoded by the nuclear genome, translated on cytoplasmic ribosomes, and selectively imported into the appropriate mitochondrial compartments.[24] Mitochondrial DNA is inherited maternally and each mitochondrion contains 2-10 mtDNA molecules.[18] Mitochondrial DNA has a very high mutation rate and when a mutation occurs, normal and mutant mtDNA can coexist within the same cell, a situation known as heteroplasmy.[16,24]

Mitochondria have long attracted the attention of biomedical researchers because of their role in human diseases. They are essential for ATP production and are susceptible to oxidative damage. Consequently, mitochondrial dysfunction has long been suggested to correlate with many human diseases and to be linked with the process of aging. Mitochondria are one of the major sources of reactive oxygen species (ROS), and are also highly susceptible to oxidative damage because ROS damage mitochondrial enzymes directly, cause mtDNA mutation, and alter mitochondrial membrane permeability leading to cell death. Most studies suggest that the majority of intracellular ROS produced by non-

phagocytic cells are derived from mitochondria.[25,26] Although the mitochondrial electron transport chain is very effective in the reduction of oxygen to water, there is a constant "leak" of electrons from the respiratory chain to oxygen and this results in the formation of superoxide anions. It is generally agreed that there are two main sites in the respiratory chain where superoxide anions are generated, complex I and complex III.[27,28] Dismutation of superoxide anions produces hydrogen peroxide as a secondary product and in the presence of transition metals, this can be converted to a highly reactive hydroxyl radical that can readily oxidize proteins, lipids, carbohydrates, DNA and RNA.[29] Thus ROS generated within mitochondria during respiration could lead to mitochondrial damage and may contribute to the mechanism of aging and to the pathogenesis of diseases.

Mitochondrial dysfunction and cell death

Over the past three decades, two fundamentally different forms of cell death, apoptosis and necrosis, have been defined. Necrosis is a passive process that happens when a major environmental damaging event causes irreversible cellular dysfunction. This results in cell swelling with cell membrane breakdown and release of cellular contents and is usually associated with an inflammatory response. Apoptosis, on the other hand, is a well defined active process that involves shrinkage of the cell, breakdown of cellular proteins, condensation of the nucleus, and cleavage of nuclear DNA, and typically does not cause inflammation.[30,31] Tissue homeostasis is critical for the survival of multicellular animals and this relies on the tightly controlled removal of superfluous, damaged and ectopic cells through apoptosis. Too little apoptosis can cause cancer or autoimmune diseases. Excessive cell death, through apoptosis or necrosis, can contribute to acute organ failure as well as chronic degenerative diseases such as Parkinson's disease.[4,14] Apoptosis research has undergone a change from a paradigm in which the nucleus determines the apoptotic process to one in which mitochondria are a major center of death control.[32-35] It is now clear that many different apoptotic signals converge on mitochondria. A variety of key events in apoptosis focus on mitochondria, including changes in electron transport, loss of mitochondrial transmembrane potential, altered cellular oxidation-reduction potential, release of caspase regulators, and participation of pro- and antiapoptotic Bcl-2 family proteins.[32,33,36-38]

In the three-step model of apoptosis proposed by Kroemer *et al*.,[32,37,39] three phases can be distinguished during apoptosis, an initiation phase, which is extremely heterogeneous, during which signal transduction cascades or damage pathways are activated, a decision phase, during which the cell decides to commit suicide, and a degeneration phase, during which proteins released from mitochondria cause the activation of programmed cell death through the activation of caspases and nucleases.[38-41] During the decision phase mitochondria integrate different death signals and trigger the decision to die by releasing proapoptotic proteins. The mitochondrial intermembrane space contains a number of cell death-promoting factors, including cytochrome *c*, second mitochondria derived activator of caspase/direct IAP binding protein with low pI (Smac/DIABLO), apoptosis-inducing factor (AIF) and procaspases.[33,34,42,43] The released cytochrome *c* interacts with procaspase 9, apoptotic peptidase activating factor 1 (Apaf-1), and ATP to trigger the assembly of the apoptosome which proteolytically activates caspase-9 and other "executioner phase" caspases.[44,45] When the caspases are activated they cleave a wide variety of proteins in the cell and this results in cell death via apoptosis.[46,47] The caspase-9/caspase-3 pathway is also regulated by proteins of the IAP (inhibitor of apoptosis proteins) family. One of these, X-linked inhibitor of apoptosis protein (XIAP), binds to caspase-9 and inhibits its proteolytic activity. The caspase-inhibiting effects of XIAP are antagonized by another intermembrane protein called Smac/DIABLO.[42,48] AIF is a flavoprotein normally confined to the mitochondrial intermembrane space. Once released to the cytosol, AIF translocates to the nucleus and induces nuclear chromatin condensation, as well as large scale (approximately 50 kb) DNA fragmentation in a caspase-independent fashion.[34,49]

Different signal-transducing molecules can influence the permeability of mitochondrial membranes through altering the subcellular localization of Bcl-2 protein family members.[50-55] The Bcl-2 family of proteins can be grouped into three subfamilies. The Bcl-2/Bcl-X_L subfamily tends to inhibit apoptosis by preventing the release of mitochondrial proapoptotic proteins such as cytochrome *c*,[33,56,57]

while the Bax/Bak and Bid/Bim subfamilies induce apoptosis by increasing the release of proapoptotic proteins from mitochondria.[34,37,58] Therefore, from all of these data, it is widely accepted that mitochondria are central to the process of cell death. As stated above, mitochondria are one of the major sources of ROS production. The ROS generated in situ can further cause damage to mitochondrial macromolecules which leads to mitochondrial dysfunction and eventually turns on the apoptosis mechanism. Hence it is reasonable to suggest that mitochondrial damage is involved in the process of human aging and degenerative diseases such as PD because during these processes progressive loss of physiological function due to cumulative cell death is the main pathological characteristic which eventually contributes to clinical symptoms.[59-61]

Mitochondrial dysfunction and Parkinson's disease

The direct evidence that mitochondrial dysfunction leads to clinical symptoms comes from mitochondrial diseases caused by mtDNA mutations.[16,24] Mitochondrial dysfunction due to defects in OXPHOS increases ROS production and leads to accumulated mitochondrial oxidative damage, energy insufficiency, cell dysfunction and progressive physiological dysfunction involving organs requiring a large supply of energy.[16,62-64] Neurodegenerative diseases often involve the death and cumulative loss of cells from regions of the central nervous system and a combination of mitochondrial dysfunction and increased oxidative stress is thought to contribute to the pathogenesis of these diseases.[65,66] Among the most prominent of these is Parkinson's disease.[10,65-67]

Pathologically, PD is characterized by the loss of the dopaminergic neurons of the pars compacta in the substantia nigra of the brain stem associated with intraneuronal protein aggregates called Lewy bodies.[68] Several hypotheses for the progressive and selective neurodegeneration in PD have been proposed. In the early 1980s, a group of young designer-drug abusers clinically presented with PD-like symptoms upon exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a synthetic by-product of heroin production.[69] The active metabolite of MPTP, 1-methyl-4-phenylpyridinium (MPP^+), selectively enters dopaminergic neurons via the dopamine transporter and potently inhibits mitochondrial complex I.[70] This results in increased oxidative stress, and decreased energy production, eventually culminating in neuronal damage and death. Since then, mitochondrial dysfunction and oxidative stress have been linked with PD. Lang and other researchers noted a 30 to 40% decrease in mitochondrial complex I activity in the substantia nigra pars compacta of patients with PD.[1,71] These findings provide direct evidence that mitochondrial dysfunction, especially complex I deficiency, is present in PD patients and may be an important cause of the development of this disease. The involvement of mitochondria in the process of dopaminergic neuronal death is also supported by epidemiological studies which indicated a role of exposure to pesticides, some of which are mitochondrial toxins, in the occurrence of PD and exposure to rotenone,[10,72,73] another complex I inhibitor and a natural product extracted from plant roots, which produces a similar phenotype in rodents.[67,74,75] Furthermore, several particular mtDNA polymorphisms and haplotypes have been reported to be associated with the risk of PD,[76-78] and mutations in mtDNA or in the nuclear-encoded mtDNA polymerase-G (POLG) cause PD-like symptoms.[79] Recently, the finding that the electron acceptor coenzyme Q_{10}, also a potent antioxidant, can slow the progressive deterioration of function in PD provides a further clue that decreasing mitochondrial oxidative stress may alter the progression of this disease. Therefore, mitochondrial dysfunction, resulting from genetic defects, environmental toxins, or a combination of the two, may cause oxidative modification of alpha-synuclein that leads to selective neurodegeneration by means of oxidative stress.[67]

Recent genetic findings in Parkinson's disease

Only a small fraction of PD cases (probably less than 10%) are caused by single-gene mutations. However, the identification of these rare, inherited mutations causing familial forms of PD have provided much insight into the discovery of novel proteins and pathways that are likely to be relevant in the pathogenesis of both the genetic and sporadic forms of the disease. These genes include α-synuclein, parkin, PTEN-induced putative kinase 1 (PINK1), DJ-1, and leucine-rich repeat kinase 2 (LRRK2). A major leap in the understanding of the etiology of the disease came from the identification of α-synuclein mutations in 1997, followed by discovery of muta-

tions in parkin the following year.[80,81] The demonstration that α-synuclein is the main constituent of Lewy bodies leads to the suggestion that cell degeneration may arise through the α-synuclein-related pathway, protein misfolding and aggregation. These proteins are ubiquitinated and initially degraded by the ubiquitin–proteasome system (UPS), in which parkin acting as an E3 ligase in the UPS system has a crucial role. The identification of mutations in DJ-1 (autosomal recessive, early onset) as a possible redox sensor,[12,82] and phosphatase and tensin homologue PTEN-induced kinase 1 (PINK1, a mitochondrial kinase) provided further evidence that mitochondrial dysfunction and oxidative stress might have a primary role in the pathogenesis of PD. PINK1 encodes a putative serine/threonine kinase with a mitochondrial targeting sequence.[12] Studies have revealed that PINK1 is associated with the inner mitochondrial membrane and is exposed to the intermembrane space.[83,84] Parkin localizes predominantly to the cytosol but also associates with the mitochondrial outer membrane. PINK1 might modulate the activity or stability of Parkin either within the mitochondrion or in the cytosol, as it might be released from mitochondria under certain conditions.[15] Moreover, in Drosophila, it has been shown that PINK1 and parkin function, at least in part, in the same pathway. These studies suggest a role for PINK1 in normal mitochondrial function and imply that parkin is downstream of PINK1.[85,86] The findings that the PINK1/parkin pathway promotes mitochondrial fission and/or inhibits fusion in Drosophila and that the loss of mitochondrial integrity in PINK1 and parkin mutants derives from reduced mitochondrial fission further support the possibility that PINK1 and parkin might regulate mitochondrial dynamics.[87,88] There is also evidence that these recessively-inherited genes (parkin, PINK1, DJ1) might all have neuroprotective effects against the development of mitochondrial dysfunction, although the exact site of their action remains unknown.[89] Therefore, it is feasible to suggest that dysfunction of these pathways results in oxidative stress ultimately leading to irreversible cellular damage and death. Consistent with genetic studies of PD around the world, researchers in Taiwan have also contributed to the research on PD by reporting several unique PD related genomic mutations including PINK1,[90,91] and LRRK2.[91-95]

The life or death decisions of dopaminergic neurons modulated by mitochondrial biogenesis and mtDNA maintenance under oxidative stress

The role of mitochondria is not only as simple powerhouses of the cell, but also as key sites and important integrators of regulatory signals affecting the cell's fate–division, growth, differentiation, survival and apoptosis.[96,97] Mitochondrial functions are performed in concert with other cell compartments and are regulated by various extracellular and intracellular signals. A host of nuclear receptors and other nuclear transcription factors, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), activator protein-l (AP-1), cAMP response element binding protein (CREB) and protein 53 (p53), involved in growth, metabolic and developmental processes, have been detected in mitochondria.[98] Recently, there has been gradually increasing support from researchers that these mitochondrially localized transcription factors can act not only on the nucleus but also on mitochondrial transcription. To adapt to a changing environment, it is necessary for cells to be able to regulate transcription of genes serving a common function by way of interaction with common binding sites in the two genomes including nuclei and mitochondria. Oxidative stress and the redox state are also involved in the survival signaling pathway of stressed cells.[99] In response to increased oxidative stress, there may be some alteration in dopaminergic neurons in terms of mitochondrial abundance, copy number and integrity of mtDNA under these pathological conditions. Within its tolerable threshold, ROS may induce protective responses through expression of specific genes to help these cells cope with hazardous environments. Once beyond this threshold, ROS may cause damage to mtDNA and other biomolecules of the affected dopaminergic neurons and elicit an apoptotic cascade of these cells by induction of mitochondrial membrane permeability transition and release of proapoptotic proteins such as cytochrome *c*, which eventually leads to dopaminergic neuron degeneration in the pars compacta of the midbrain. Being the major supplier of energy in mammalian cells, mitochondria are necessary to provide more energy for damage repair and cellular survival during disease processes with excessive oxidative stress. In order to meet the demand for energy supply, signals transmitted to the

nucleus may induce mitochondrial proliferation and mtDNA amplification to produce more functional mitochondria. The abundance of mitochondria in a cell is determined by the biogenesis and division of the organelles and tightly controlled by the activation of specific transcription factors encoded by nuclear genes.[100] Nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) are transcriptional regulators that act on the nuclear genes coding for peptides necessary for the mitochondrial respiratory system. They also regulate the expression of many other genes involved in mtDNA replication.[101,102] Mitochondrial transcription factor A (Tfam) acts on the promoters within the D-loop region of mtDNA and it regulates the replication and transcription of the mitochondrial genome.[103] Both NRF-1 and NRF-2 can regulate the expression of the Tfam gene by binding to the consensus-binding sites. This provides a unique mechanism for the cell to integrate the expression of nuclear DNA-encoded proteins with the transcription of genes encoded by mtDNA (Fig. 1).[103] Recently, much evidence has emerged that peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) is a major regulator of mitochondrial biogenesis.[104] PGC-1α regulates the expression of transcription factors involved in the coordinated expression of mitochondrial genes, such as NRF-1 and NRF-2, which in turn trigger the expression of

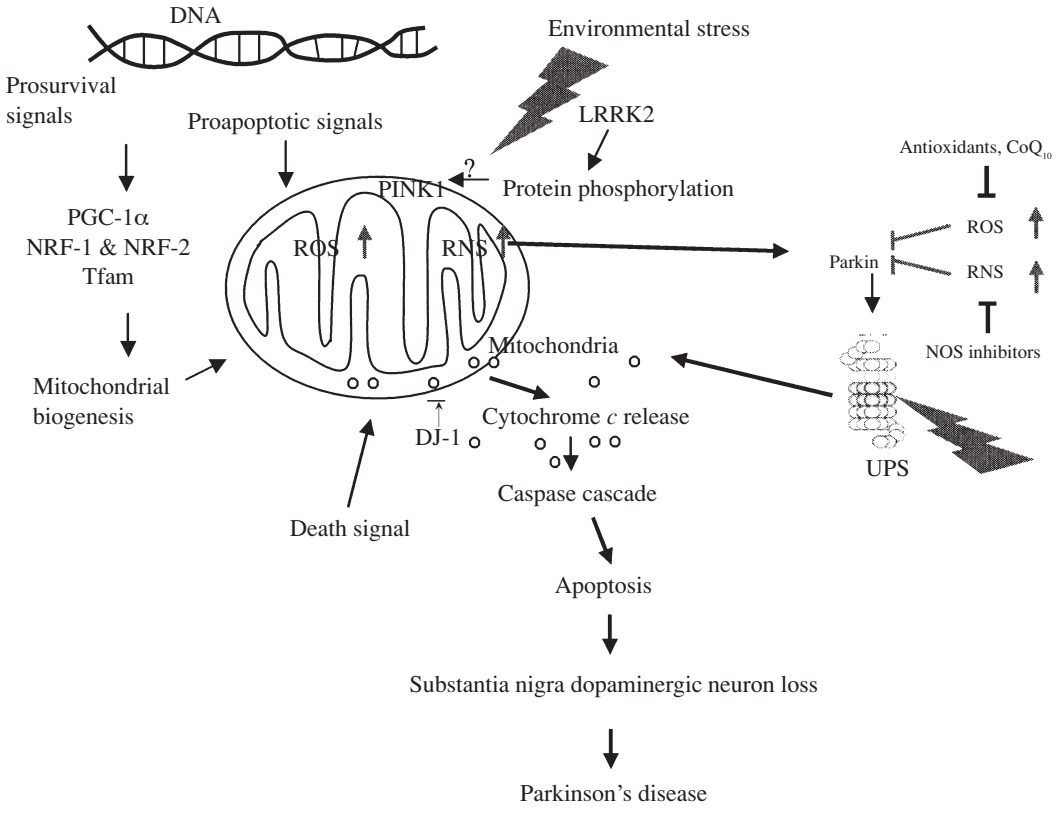

Fig. 1 Schematic representation of possible mechanisms involved in dopaminergic neuron degeneration. Multiple factors inducing genetic variation including PINK1, DJ-1, Parkin, and possibly LRRK-2 and environmental toxins may modulate the process of life or death decision making by neurons. Mitochondrial dysfunction from various causes can lead to the signaling of mitochondrial biogenesis and mitochondrial oxidative stress, and regulate the process of cell death signaling which eventually leads to degeneration of dopaminergic neurons in the substantia nigra of the midbrain and contributes to the development of PD. Mutations in PINK1 lead to mitochondrial dysfunction and, in combination with LRRK2 mutation may lead to abnormal phosphorylation of proteins, possibly including mitochondrial proteins. Parkin is downstream of PINK1 and might itself cause mitochondrial abnormalities in addition to impaired ubiquitination of proteins. DJ-1 can act as an antioxidant, and mutations in DJ-1 are associated with oxidative stress and possibly cellular apoptosis.

nuclear genes coding for polypeptides of the respiratory chain and proteins involved in transcription and replication of mtDNA.[104] However, there are few studies clarifying the involvement of mitochondrial biogenesis in the life-and-death decision making of the neurons. The report that a genetic variant rs2306604 A-allele in Tfam could be a moderate risk factor for Alzheimer's disease (AD) suggests that disturbance of maintenance of mtDNA integrity or mitochondrial function may underlie neurodegenerative disorders.[105] Recently, the production of a Tfam knockout mouse model of PD and the reduced mtDNA expression and respiratory chain deficiency in the midbrain dopaminergic neurons of these animals further support this hypothesis.[106] Therefore, to prevent the death of environmentally stressed neurons, a thorough understanding of pro-survival pathways of the striatal neurons may be crucial for the prevention of disease progression.

Conclusion

Evidence has been presented from various experimental studies that impairment of mitochondrial function may be involved in the pathological process culminating in neuronal cell death in PD. Preventing mitochondrial dysfunction or restoring mitochondrial biogenesis might block the pathological process at an early stage. Understanding the interaction between these different mechanisms involving life or death decisions during the process of disease development might offer novel prospects for therapy based on targeted neuroprotection of vulnerable neurons. Further therapeutic interventions can be envisaged, including strategies to render cells more resistant to conditions associated with mitochondrial dysfunction, application of antioxidants to protect the neurons from oxidative damage and treatments to maintain proper mitochondrial biogenesis in the early stage of disease.

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粒線體功能異常及生物合成於巴金森氏病之病因論述

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巴金森氏病是一種較常在老年人出現的神經系統退化性疾病。神經病理上的特徵包括黑質紋狀體的多巴胺分泌退化，以及細胞內有被稱爲路易體 (Lewy bodies) 的不正常蛋白質沉積。目前巴金森氏病雖然有藥物可以使病狀獲得相當程度的改善，但是此病仍然無法「痊癒」，長期的慢性病程，病患的病情仍會逐漸惡化，甚至於會到與床爲伍的地步。直到現在，此病的致病機轉仍然是個謎。經過了許多神經科學家多年的努力，我們已經了解在巴金森氏病患者的腦組織可以發現粒線體功能異常、細胞受到氧化壓力以及發生細胞凋亡。因爲粒線體目前已知是細胞生存以及退化死亡的重要關鍵胞器。最近發現會引起家族型巴金森氏症的基因如PTEN-induced putative kinase 1 (PINK1)，DJ-1，α-synuclein，leucine-rich repeat kinase 2 以及 parkin—都被研究顯示與粒線體功能有關連，同時這些基因也牽涉到氧化壓力及自由基所引起的細胞傷害。這些發現都強化了粒線體與巴金森氏病的密切關連性。在不良環境下的神經細胞，爲了生產更多的能量以求活存，也會加強粒線體質與量的生物合成。因此進一步的探討神經細胞在因應不良環境時所發生的求生機轉，如粒線體生物合成及死亡機轉如細胞凋亡，我們將可能調控細胞的求活與凋亡機轉，進而運用這些結果於臨床上。這些知識也許將能被使用來減緩此一神經退化疾病進展的速度，並且提供未來尋求根本治療巴金森氏病的方針。(長庚醫誌2009;32:589-99)

關鍵詞：巴金森氏病，粒線體功能異常，粒線體生物合成，基因，細胞存活與死亡

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