Mitochondrial Signaling through PTEN-Inducible Putative Kinase-1 (PINK1) in Response to Ischemia: Lessons from Familial Parkinson's Disease

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Abstract

While much is known about the signaling events elicited in response to stroke, the role of mitochondrial signaling following an ischemic event has only recently begun to be investigated. Increasing evidence demonstrates mitochondrial dysfunction in numerous neurological disorders including Parkinson's disease (PD), Alzheimer's disease, Huntington's disease, autism spectrum disorders and stroke, which suggests that understanding the components of mitochondrial signaling in these disorders may uncover conserved signaling molecules at the level of the mitochondria. The recent identification of genetic mutations that result in the development of familial forms of PD and affect mitochondrially-associated proteins, such as the stress-associated kinase PTEN-Inducible putative kinase 1 (PINK1) and the E3 ubiquitin ligase Parkin, have provided new and interesting insights into mitochondrial signaling pathways in response to stress. Studies regarding the PINK1 pathway suggest a role for this protein in mitochondrial quality control via initiation of the selective autophagic removal of damaged mitochondria or mitophagy. Given that mitophagic processing has been shown to occur in a number of different stress paradigms suggests that PINK1 may act as global sensor of stress, recognizing damaged mitochondria in a number of pathological settings including that of neuronal ischemia. This review aims to introduce evidence for a link between PD and stroke namely through conserved PINK1 signaling at the level of the mitochondria.

Keywords

Ischemic stroke
excitotoxicity
mitophagy
PINK1
Parkin
CHIP

Stroke: The Basics

Stroke is the world's second leading cause of mortality, accounting for over 6,000,000 deaths annually[2]. Moreover, stroke is the leading cause of long-term adult disability[3] and accounts for nearly 70 billion dollars per year in direct costs such as healthcare, prescriptions and rehabilitation, as well as, indirect costs such as those that accrue from loss of workforce and therefore loss of economic efficiency[4]. Ischemic stroke encompasses 85% of stroke cases and is defined as an occurrence in which a reduction in blood flow results in alterations in normal cellular function[2,5]. The major risk factors for ischemic stroke include age, diabetes, and hypertension— all of which are represented though family history and genetics[6]. While these risk factors account for a significant portion of strokes, there is no explanation as to why some patients with similar risk profiles incur strokes while others do not[7]. Currently the estimated lifetime risk for stroke lies between 8 and 10%[2], yet both preemptive treatments for high-risk patients , as well as therapies that could reduce neuronal damage following a stroke remain elusive[4].

Ischemic Stroke: Pathophysiological Mechanisms

A blocked cerebral vessel results in decreased supply of oxygen and glucose to an area or areas of the brain that usually rely on blood flow from the occluded vessel. These events trigger the initiation of what is often referred to as an excitotoxic cascade wherein the loss of oxygen and glucose leads to a subsequent loss of energy in the form of ATP. As such, neurons in this region no longer have the proper substrates to carry out oxidative phosphorylation and instead are forced to switch to anaerobic respiration. Without ATP, numerous energy-dependent membrane pumps become dysregulated, the neuron becomes depolarized and an influx of calcium ions (Ca^{2+}) results[8]. Because ATP-dependent ion pumps can no longer remove the Ca^{2+} from the cell, intracellular calcium becomes much higher than physiological levels. This increased intracellular calcium initiates two major events: activation of enzymes, proteases and endonucleases that further disrupt the cellular membrane and the release of the major excitatory neurotransmitter, glutamate. The presence of excessive amounts of glutamate in the synaptic cleft causes stimulation of both AMPA receptors and Ca^{2+} permeable NMDA receptors on neighboring neurons, evoking even more Ca^{2+} to enter these already damaged neurons. The resulting overexcitation leads to the generation of free

radicals, specifically reactive oxygen species (ROS[a]), as well as further glutamate release. In addition, phospholipases continue to be activated and ,consequently, excessive membrane damage and non-regulated movement of ions into and out of the cell occurs. The overall result of excess intracellular calcium, excess glutamate release, the generation of free radicals and ROS, in addition to the breakdown of the cell's outer membrane is termed neuronal excitotoxicity[b] and often results in neuronal cell death[9].

Ischemic Stroke: Understanding the Role of Mitochondria

An ischemic event in the brain is particularly devastating due to this organ's high metabolic demand and, therefore, its major reliance on mitochondria[10]. The excitotoxic cascade is initiated in large part by loss of ATP generation within mitochondria, which are the site of greater than 90% of ROS generation— yet another major consequence of ischemia. In addition to their role in maintaining cellular energetics, mitochondria are also intimately involved in the regulation of cellular ion homeostasis and are particularly well known for their role in caspase-dependent apoptosis, often the end result of ischemic events. Given the connection between mitochondria and the events involved in the excitotoxic cascade, identifying the molecules and molecular complexes involved in the ischemic response, as well as understanding their mechanisms of action at the level of these organelles, is of utmost importance.

Mitochondrial signaling in response to stress has gained much attention in relation to diseased states of the brain. In fact, many recent articles have focused on the role of abnormal or dysregulated mitochondria in neurodegenerative diseases including Parkinson's Disease (PD), Alzheimer's Disease (AD) and typical ageing processes[10,11]. In this context, major strides have been made in identifying genetic mutations in mitochondrial proteins in the PD field. Because there are a limited number of molecules available to participate in mitochondrial signaling, it is plausible that crucial insight can be gained from the Parkinson's Disease literature regarding mitochondrial responses to stress.

Parkinson's Disease: Basic Information and an Introduction to Genetic Mutations

Currently, Parkinson's disease is estimated to effect 6 million people worldwide, although many undiagnosed cases are probable. PD is therefore noted as the most common age-related neurodegenerative movement disorder (WHO). PD patients typically present with bradykinesia, resting tremor, muscular rigidity and postural instability, as well as, major cellular hallmarks such as the presence of cytoplasmic Lewy bodies and neuronal cell loss specifically within the substantia nigra pars compacta[12]. The majority of PD cases are sporadic, exhibiting no genetic inheritance; however, 5% of PD cases are familial[12]. Recently, genetic links have been discovered in autosomal recessive forms of PD and include mutations in the PARK2[c] and PARK6[d] genes[12-15]. One of the most interesting findings regarding single mutations in either PARK2 or PARK6 is that mitochondrial turnover is dysregulated[16].

PINK1 and Parkin: Involvement in Mitochondrial Quality Control and Mitophagy

PINK1 is a ubiquitously expressed, 63kDa protein that is encoded by the PARK6 gene. It has a N-terminal mitochondrial targeting sequence (MTS) that is inserted into the outer mitochondrial membrane (OMM), as well as a C-terminal kinase domain that faces the cytosol. When directed to healthy mitochondria via its MTS, PINK1 inserts into the OMM and is immediately cleaved by mitochondrial proteases and released into the cytosol where it is delivered to the proteasome for degradation[17-19]. Ongoing studies that focus on the mechanisms of PINK1 cleavage have identified a number of mitochondrial proteases that cleave PINK1. Examples of these proteases include presenilin-associated rhomboid-like protein (PARL) and matrix metalloproteinase (MMP), both of which have been shown to generate markedly different PINK1 cleavage products. Such studies are key as they may uncover novel signaling roles of the various PINK1 cleavage products. Furthermore, when mitochondria is injured or depolarized, PINK1 not only becomes stabilized but also accumulates in the OMM due to the inhibition of mitochondrial proteases[19]. In such cases, PINK1 then acts to recruit the 53kDa, cytoplasmic, E3 ubiquitin ligase, Parkin, to damaged mitochondria[18]. Extensive studies by Matsuda, et al have demonstrated that while in the cytosol, the ubiquitin ligase activity of Parkin is repressed; however, once stabilized at the mitochon-

a. **Reactive Oxygen Species (ROS)**: Normal byproducts of mitochondrial metabolism that increase dramatically during times of cellular stress.

b. **Excitotoxicity**: A pathological process during which neurons are severely damaged due to excessive stimulation by neurotransmitters often resulting in neuronal cell death.

c. **PARK2**: Gene encoding the E3 ubiquitin ligase, Parkin, found to be mutated in 50% of autosomal recessive forms of PD as well as 10-15% of sporadic PD cases.

d. **PARK6**: Gene encoding the stress-associated kinase, PINK1, found to be mutated in some cases of familial Parkinson's disease.

dria, via recruitment and interaction with PINK1, its enzymatic activity is unmasked[18]. Activated Parkin has been shown to ubiquitinate protein substrates of mitochondria with reduced membrane potential following treatment with the mitochondrial uncoupling agent, carbonyl cyanide m-chlorophenylhydrazone (CCCP)[18]. Furthermore, using mouse embryonic fibroblasts (MEFs) from PINK1 wildtype (WT) and PINK1 knockout (KO) mice, it has been demonstrated that only WT MEFs are able to recruit Parkin to mitochondria following CCCP treatment and, following this recruitment, damaged mitochondria are cleared from the cells[18]. The disappearance of mitochondria following CCCP treatment was also noted by another group when they compared HeLa cells lacking Parkin to HeLa cells expressing Parkin. This study found that 48 hours following treatment, Parkin expressing HeLa cells had no remaining detectable mitochondria as assessed by three independent mitochondrial markers[20]. Additionally, following administration of CCCP the knockdown of an essential mammalian autophagy protein, autophagy-related protein 7 (Atg7), in Parkin-expressing HeLa cells demonstrated a loss of damaged mitochondrial clearance[20, 21].

Together, these data support a hypothesis whereby PINK1 accumulation and stabilization within the OMM of a damaged, depolarized mitochondria leads to the recruitment of Parkin from the cytosol to these organelles where its unmasked E3 ligase activity, results in the ubiquitination of mitochondrial substrates and, in turn, orchestrates the selective autophagy of damaged mitochondria, otherwise known as mitophagy[e*] (**Figure 1**).

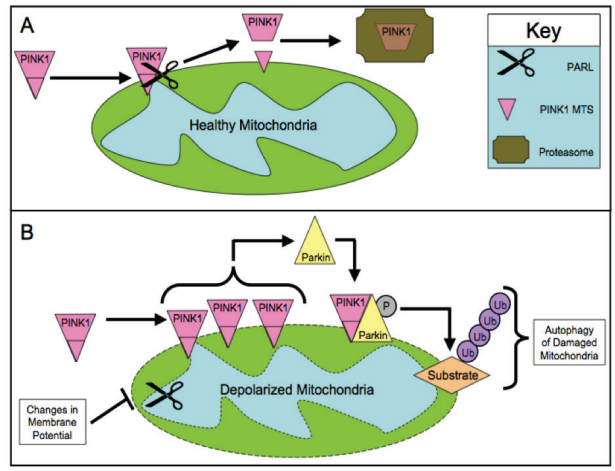

Figure 1: PINK1 plays a key role in determining healthy versus damaged mitochondria. Healthy mitochondria (A) undergo constitutive turnover of PINK1 via the proteasome. Damaged, depolarized mitochondria (B) accumulate PINK1 in their OMM which leads to the subsequent recruitment of other molecular players necessary for autophagy of unhealthy mitochondria also known as mitophagy (adapted from [1]).

Ischemic Stroke: Mitophagic Responses and the Role of E3 Ligases

Autophagy commonly refers to the bulk degradation of the cytoplasm and organelles in order to regulate intracellular homeostasis and numerous groups have reported changes in autophagy in response to different types of *in vivo* and *in vitro* stress paradigms including ischemic models (Reviewed in[22]). Recently, we have become aware of more selective forms of autophagy that target specific organelles for degradation such as mitochondria. In an elaborate set of experiments, Narenda et al., demonstrated for the first time that Parkin is a regulator of the selective mitophagic response[20]. Another E3 ligase, carboxy-terminus of HSC70 interacting protein (CHIP), has been shown to enhance Parkin activity under normal circumstances and to compensate for loss of Parkin activity in cases of Parkin mutation[23]. Interestingly, Stankowski et al., have shown that CHIP expression levels are increased in post mortem human brain tissue samples from patients that had suffered from either a transient ischemic attack (TIA) or a stroke. In addition, they found that CHIP is also upregulated in response to oxygen and glucose deprivation (OGD) in an *in vitro* model of primary rat cortical neurons[24]. The noted interactions between CHIP and Parkin are interesting in light of the emerging role of CHIP in ischemia. Given the previously discussed data supporting PINK1 involvement in the recruitment of Parkin and subsequent mitophagic processing, a closer investigation into the possible role of the PINK1 pathway in response to ischemia and in relation to CHIP is warranted.

Ischemic Stroke: Involvement of PINK1 Signaling

Thus far only one study has been published with regards to a role for PINK1 in cerebral ischemic models. This study demonstrates a decrease in PINK1 expression in primary cortical neuronal cultures 24 hours following 2 hours of OGD[25]; however, the cells utilized were only cultured for 12 days *in vitro* (DIV) while the literature supports that mature NMDA receptors expressing the necessary subunits to respond to excitotoxicity are not developed until at least

e. **Mitophagy**: The selective, autophagic degradation of damaged mitochondria that can occur as a means to regulate mitochondrial quality control under normal cellular conditions or in response to cellular stressors.

DIV14[26]. In addition, given that PINK1 is immediately targeted to mitochondria via its MTS and that the previously discussed studies find few remaining damaged mitochondria by 24 hours following a stress due to mitophagic clearance[18, 20], it may be that earlier time points following OGD need to be examined. Accordingly, a study of ischemia in spinal cord neurons demonstrated, via Western blot and immunocytochemistry, increases in PINK1 expression in response to this stress within 8 hours[27].

Given the immense amount of new evidence supporting a role for PINK1 and Parkin in general mitochondrial quality control mechanisms[28,29], the heavy reliance of neurons on mitochondrial support and the increasingly noted affect of mitochondrial dysfunction in neurodegenerative diseases, more detailed investigations into the role of mitochondrial signaling in response to ischemic events may uncover novel therapeutic targets for the treatment of stroke.

Conclusion:

Stroke and Parkinson's disease are common neurodegenerative disorders affecting millions of patients worldwide. Although science has made great strides in identifying the molecular mechanisms and pathways involved in these disorders, therapeutic intervention remains elusive. In recent years, the role of PINK1 signaling at the level of the mitochondria in response to stress has come to the forefront of research studies as we now recognize common themes regarding mitochondria and stress signaling across such disorders. As we continue to search for therapeutics for these neurological diseases, interesting new insights into the relationship between general mitochondrial dynamics and neurodegeneration have come to light. In addition, many conserved pathways and molecules have been uncovered, including but not limited to, those that involve: PINK1, Parkin and CHIP. The purpose of this review was to not only introduce these relatively new molecular players, but also to remind us of what can be learned from similar but different fields of science and to stimulate further interest and investigation of potential therapeutic targets across these fields towards a common goal.

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