Mitochondria, OxPhos, and neurodegeneration: cells are not just running out of gas

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Mitochondrial respiratory deficiencies have been observed in numerous neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases. For decades, these reductions in oxidative phosphorylation (OxPhos) have been presumed to trigger an overall bioenergetic crisis in the neuron, resulting in cell death. While the connection between respiratory defects and neuronal death has never been proven, this hypothesis has been supported by the detection of nonspecific mitochondrial DNA mutations in these disorders. These findings led to the notion that mitochondrial respiratory defects could be initiators of these common neurodegenerative disorders, instead of being consequences of a prior insult, a theory we believe to be misconstrued. Herein, we review the roots of this mitochondrial hypothesis and offer a new perspective wherein mitochondria are analyzed not only from the OxPhos point of view, but also as a complex organelle residing at the epicenter of many metabolic pathways.

Introduction

The accumulation of mutations in mitochondrial DNA (mtDNA) and subsequent reductions in mitochondrial oxidative phosphorylation (OxPhos) are thought to occur during the course of neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) (1). Since we are often looking for the answer where we see clues, it should not come as a surprise that over the years a large body of literature has led to the controversial, but nevertheless popular, hypothesis that mitochondrial defects are instrumental in provoking neuronal death in common adult-onset neurodegenerative disorders.

When mitochondria are studied in isolation from the rest of the cellular machinery, and from the traditional bioenergetic point of view, their role in the regulation of overall cellular homeostasis is usually overlooked. Mitochondria are not only "powerhouses" that generate energy via OxPhos but also integrate numerous cellular signaling and metabolic pathways (2) that have only recently been considered in the study of disease. Alterations in these pathways can result in mitochondrial dysfunction that, in turn, can affect OxPhos output, rather than vice versa.

Thus, while the alterations in mitochondrial respiration reported in many neurological conditions (Tables 1–3) may truly reflect a primary OxPhos defect, they might also be mere bystander consequences of non–OxPhos-related cellular problems. Furthermore, detection of an OxPhos defect often leads the disorder to be labeled a mitochondrial disease that is conceptually no different from a classic OxPhos disease (see below). Yet, could it be that altered OxPhos is a metabolic feature of many disorders without necessarily being etiologically or pathogenically significant? The question, incidentally, applies not only to common neurodegenerative disorders (Table 1) such as AD and PD, but also to other neurological conditions (Tables 2 and 3) such as very common peripheral neuropathies (Table 3), including those induced by chemotherapeutic agents and HIV as well as those associated with diabetes that are reported to comprise a mitochondrial component (3). The task of deciphering the role of mitochondria in neurodegeneration is further complicated by the fact that enhancing mitochondrial respiration within compromised neurons may well be beneficial even if the actual disease mechanism is not OxPhos-dependent.

In this Review, we first provide a brief historical context for how the mitochondrial OxPhos hypothesis of neurodegeneration emerged. We then take the provocative position that the links between adult-onset neurodegenerative disorders and OxPhos have been misconstrued. Finally, in light of newly published data, we offer an alternative explanation as to how mitochondrial deficits may participate in the neurodegenerative process.

Reduced OxPhos and ATP production in neurological conditions

Before discussing potential links between mitochondria and neurodegeneration, we need to clarify what is an "authentic" mitochondrial disease due to OxPhos deficiency. Primary mitochondrial disorders result from mutations in nuclear DNA or mtDNA that encode subunits of the OxPhos system or factors involved in its expression and assembly (4). Many primary mitochondrial disorders present with a range of neurological manifestations, and, conversely, neurological conditions highly suggestive of being due to a mitochondrial problem typically include a relapsing-remitting pattern, with incremental worsening and partial recovery; an age at onset prior to 40 (generally during childhood); a maternal pattern of inheritance; a multisystem presentation, reflecting the involvement of different parts of the nervous system and frequently affecting other organs; and the presence of lactic acidosis in blood and cerebrospinal fluid as well as mitochondrial proliferation in skeletal muscle (ragged-red fibers). Furthermore,

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Table 1. Main clinical syndromes reported to be associated with OxPhos deficiency

Neurological disorder	Typical clinical presentation	Main neuropathological features
Alzheimer's disease	Cognitive impairment primarily featuring memory problems; as the disease progresses, language, perceptual skills, attention, constructive abilities, orientation, problem solving, and functional ability difficulties also arise, as well as behavioral and neuropsychiatric changes, including wandering, irritability, and labile affect	Cerebral cortex atrophy associated with amyloid plaques and neurofibrillary tangles and gliosis
Parkinson's disease	Association of shaking, slowness of movements, stiffness, and poor balance	Loss of pigmented neurons in ventral midbrain (e.g., substantia nigra pars compacta) and other pigmented nuclei (e.g., locus caeruleus, dorsal motor nucleus of the vagus); intraneuronal Lewy body inclusions; gliosis
Amyotrophic lateral sclerosis	Muscles weakening, wasting away, and twitching; increased muscle tone, brisk reflexes	Loss of cortical and spinal motor neurons; degeneration of corticospinal tract; multiple forms of proteinaceous inclusions; gliosis
Huntington's disease	Uncontrolled movements (called chorea), abnormal body postures, and changes in behavior, emotion, judgment, and cognition	Atrophy of caudate nucleus and putamen accompanied with mild frontal and temporal atrophy; loss of medium-size spiny neurons in the striatopallidal and striatonigral pathways associated with striatal gliosis
Charcot-Marie-Tooth disease	Progressive muscle wasting and weakness; sensory loss	Loss of large myelinated motor and sensory fibers in peripheral nerves

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even if cognitive decline, parkinsonism, ataxia, or other common signs of neurodegenerative disorders occur in authentic mitochondrial diseases, they are almost never observed in isolation, in contrast to prototypical neurodegenerative disorders. In rare instances in which parkinsonism was indeed observed in patients with mtDNA mutations, atypical PD features, such as deafness and peripheral neuropathy, were also noted (5).

It is increasingly recognized that identical molecular abnormalities may give rise to heterogeneous clinical and neuropathological phenotypes, as illustrated by the tauopathies (6) and leucine-rich repeat kinase 2 (*LRRK2*) mutations (7). This raises the possibility that adult-onset neurodegenerative disorders might indeed be authentic mitochondrial disorders that simply do not fit into the canonical mold of mitochondrial disease depicted above.

Table 2. Other suspected mitochondria-related diseases

Neurological disorder	Typical clinical presentation	Main neuropathological features
Leigh's syndrome	Progressive loss of mental and movement abilities (psychomotor regression) with high serum lactic acid levels	Bilateral symmetrical lesions in the brainstem and basal ganglia with gliosis, vacuolation, capillary proliferation, relative neuronal preservation
Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)	Episodes of muscle weakness on one side of the body (hemiparesis), altered consciousness, vision abnormalities, seizures, and severe headaches; can progress to vision loss, problems with movement, and dementia	Multifocal infarct-like lesions in the posterior cortex; recurrent stroke-like episodes; lactic acidosis and ragged-red fibers
Kearns-Sayre syndrome	Progressive external ophthalmoplegia, retinitis pigmentosa; common additional features include deafness, cerebellar ataxia, and heart block	Loss of neurons and gliosis of the basal ganglia; spongy change of the white matter in the cerebrum, brain stem, and cerebellum; retinal degeneration; muscle ragged-red fibers
Optic atrophy	Bilateral visual loss; central vision affected prior to peripheral vision	Degeneration of the retinal ganglion cell bodies and axonal pathways up to the lateral geniculate nuclei
Friedreich's ataxia	Incoordination of gait and often of hands; other features include gradual loss of strength and sensation in the arms and legs, muscle stiffness (spasticity), and impaired speech, hearing, and vision; often the heart is also affected	Thinning of dorsal roots, degeneration of dorsal columns, atrophy of the neurons in the Clarke's column and dorsal spinocerebellar fibers, atrophy of gracile and cuneate nuclei, and neuropathy of sensory nerves; lesions of the dentate nucleus and the corticospinal tracts are also observed
Spinocerebellar ataxia	Incoordination of gait and often of hands, speech, and eye movements	Degeneration of the spinal cord and the cerebellum, as well as many nuclei of the basal ganglia and the brainstem
Primary coenzyme Q_{10} deficiency syndrome	Syndrome affecting brain (incoordination and poor balance), muscles (weakness), and kidney (nephrotic syndrome); other neurological abnormalities include seizures, intellectual disability, hypotonia, dystonia, spasticity, nystagmus, vision loss, and deafness	Multisystem neurodegeneration with gliosis
Progressive encephalopathy associated with cytochrome *c* oxidase deficiency	Progressive uncoordinated gait, dysarthria, lower limb areflexia, deafness, and high serum lactic acid levels, followed by dementia	Cortical atrophy and basal ganglia calcifications as well as severe mitochondrial myopathy with numerous COX-negative ragged-red fibers
Hereditary spastic paraplegia	Difficulty in walking and poor balance followed by increased muscle tone, brisk reflexes, muscle weakness, bladder disturbances, and paresthesia	Degeneration primarily in the corticospinal tracts and the fasciculus gracilis, and to a lesser extent in the spinocerebellar tract

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Table 3. Mitochondria-related neuropathies

Neurological disorder	Typical clinical presentation	Main neuropathological features
Chemotherapy-induced peripheral neuropathy	Burning/shooting pain, tingling, cramping, and weakness in hands and feet; impaired balance and walking, falls; loss of heat sensitivity	Peripheral nerve neuronopathy and/or myelinopathy; axonopathy, particularly intraepidermal nerve fiber degeneration; there are differences in the pathobiology depending on the drug used
Diabetic neuropathy	Sensory impairment with loss of sensory function or spontaneous feeling of touch, vibration, pricking, and hot and cold pain; bilateral and symmetric damage to nerves of the feet and hands	Retrograde neurodegenerative disease of the peripheral nervous system; damage to the small sensory nerve fibers accompanied by continuous and episodic pain
HIV-induced neuropathy	Debilitating chronic neuropathic pain that is constant and severe; bilateral pain on the soles; the dysesthesias ascend to lower extremities and may involve fingertips	Retrograde axonal degeneration of long axons in distal regions of legs or arms, loss of unmyelinated fibers, and macrophage infiltration in peripheral nerves and dorsal root ganglia

Therefore, if the clinical presentation is of little help in clarifying the link between mitochondria and neurodegeneration, can genetics be a more fruitful approach?

Efforts to find specific mtDNA alterations in PD yielded confusing results, as some groups demonstrated the existence of mtDNA mutations, including partial deletions, in patients with parkinsonism (8–13) whereas others found few or none (14–17). Sequencing of aged healthy individuals showed the accumulation of somatic mtDNA mutations, leading to the proposal that mtDNA point mutations in PD patients rendered their mitochondria susceptible to failure (18, 19). Data from other groups, however, challenged these results (20). Likewise, the quest for mtDNA candidate genes in PD revealed a significant risk associated with the A4336G mutation in mt-tRNA^{Glu} (21), which was challenged by others (22, 23). Lastly, specific mtDNA haplotypes conferring susceptibility to PD were reported (24), but how much of a role these gene variants play in the occurrence of PD remains to be established.

A search for AD-specific mtDNA mutations was equally inconclusive (25–27). An early report identified a point mutation in the NADH dehydrogenase 2 (ND2) subunit of complex I (CI) in brain tissue of 19 AD patients (28), despite the lack of evidence for maternal inheritance in familial AD (29). These results were not replicated by others (30), nor were reports of mtDNA mutations (31, 32) in mt-tRNA^{Gln} (33) and in subunits I and II (COX I/II) of complex IV (CIV; also known as cytochrome *c* oxidase) (34). Also, the ND2 mutation is not present solely in AD (28), implying that it is likely a neutral polymorphism (35). Some groups found elevated levels of the relatively common mtDNA 4977-bp deletion (36) in AD brain compared with controls (37, 38), but this deletion has also been associated with aging (39) and other neurodegenerations, such as amyotrophic lateral sclerosis (ALS) (40). Similarly, reports of reduced mRNA levels for mitochondrial CI and CIV subunits in AD brains (41–44) were not correlated with the appearance of disease phenotypes (45). More recent studies have corroborated the increased presence of mtDNA partial deletions (46, 47) and point mutations (48), altered mtDNA methylation (49), and reduced expression and activity of respiratory chain complexes (50) in AD.

It may thus be concluded from the above that even if future studies unequivocally link mutations in mtDNA to neurodegenerative disorders such as AD and PD, they do not appear to be primary causes and may even reflect nonspecific changes in dying neurons or result from technical artifacts (51). Another possibility as to why findings of mtDNA mutations in neurodegenerative disorders have been inconsistent is that they may be acquired in the course of the disease as a consequence of a prior specific insult. For example, an initial error might cause defects in the replication and repair of mtDNA (52), resulting in genome instability and the appearance mtDNA defects in the course of neurodegeneration (53, 54). As in the aging process (27, 55–57), these mutations would accumulate in cells until they crossed a threshold beyond which decline in bioenergetics emerges (58, 59). Thus, given that different tissues and cells have different thresholds and compensatory mechanisms (58, 60), understanding mitochondrial defects in neurodegenerative disorders as consequences instead of as initiators would help explain not only some of the contradictory results obtained by different groups (61), but also the differences in the clinical presentations as compared with authentic OxPhos disorders (62).

Common neurodegenerative disorders and the mitochondrial hypothesis

If common neurodegenerative disorders depart clinically from authentic mitochondrial diseases, how did the hypothesis arise that a causal link might exist between adult-onset neurodegeneration and mitochondria? Historically it is likely that the first proposal of such a connection involved PD. In the early 1980s, contemporaneously with the identification of a number of mitochondrial syndromes, it was discovered that exposure to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) via illicit drug use led to an acute parkinsonian syndrome that was clinically indistinguishable from PD (63). This PD-like condition was shown to result from blockade of mitochondrial electron flow at the level of CI (64, 65). Subsequently, reports that MPTP (66) and other CI inhibitors (67–69) produced some of the features of PD in rodents strengthened the idea that CI inhibition can cause PD-like neurodegeneration. The similarity between MPTP-induced parkinsonism and PD led investigators to assess mitochondrial respiration in biospecimens from PD patients. They found significant reductions in respiratory chain activity in PD tissues (70), particularly in CI in PD brain (71, 72) and platelets (73, 74), as well as structural alterations in CI subunits (75), leading to the conclusion that deficient CI function was key to PD pathogenesis. However, other results cast doubt on this view, as

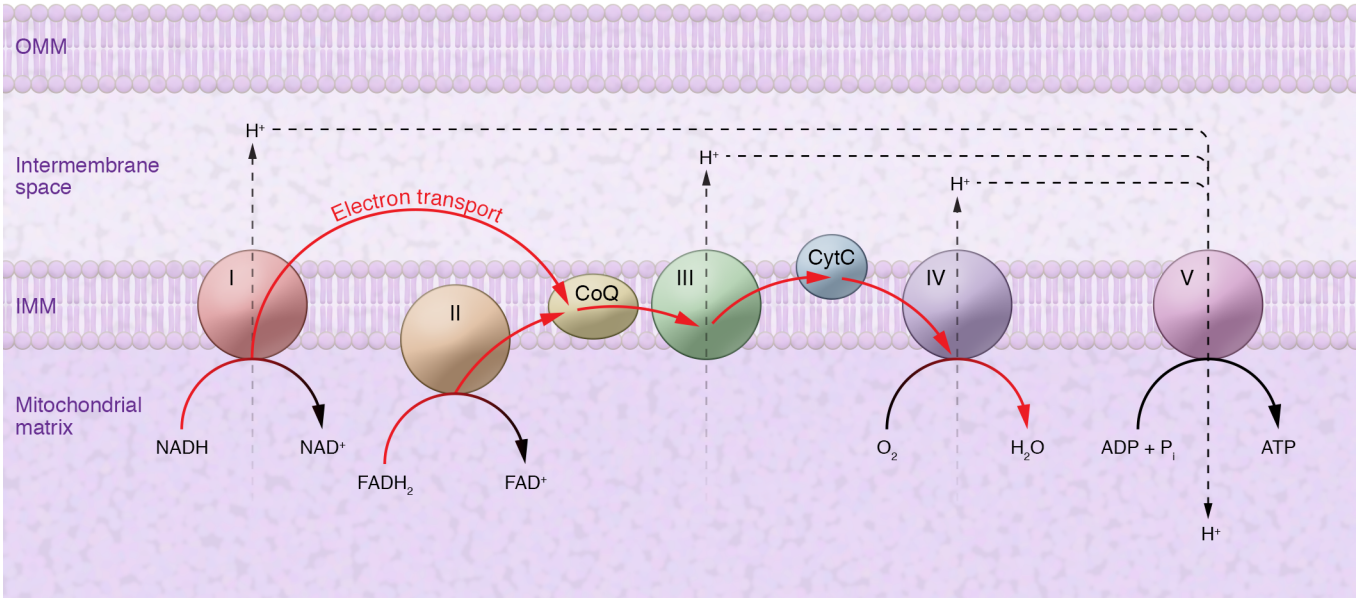

Figure 1. Simplified model of mitochondrial aerobic energy production (stoichiometries not implied). The mitochondrial electron transport chain (ETC) is composed of enzymatic complexes (I–V) that transfer electrons from electron donors (NADH and FADH2) to electron acceptors embedded in the IMM via redox reactions, ultimately generating water. The ETC couples electron transport to the transfer of protons (H^+) across the IMM, creating an electrochemical proton gradient that drives the synthesis of ATP by ATP synthase. OMM, outer mitochondrial membrane. CytC, cytochrome *c*; CoQ, coenzyme Q_{10}.

some groups were unable to confirm CI deficiencies in PD muscle (76). Moreover, the chronic use of levodopa, a widely used anti-PD therapy, was found to alter OxPhos activity in rodent brains (77, 78). Finally, the cellular origin of reported reductions in CI activity was called into question, as postmortem brains from PD patients are mostly devoid of dopaminergic neurons, a main target of PD neurodegeneration (66).

Almost contemporaneously with the discovery of MPTP's parkinsonian effect, the connection between mitochondria and AD emerged from reports of mitochondrial morphological alterations in postmortem brain sections (79, 80) and metabolic alterations in fibroblasts from patients, such as reduced glucose and deficits in calcium homeostasis (81). Given the neuronal vulnerability to these insults, it was hypothesized that mitochondrial dysfunction underlay the behavioral deficits in AD. Furthermore, cytoplasmic hybrids (cybrids) repopulated with mitochondria from AD patients reportedly displayed mitochondrial alterations (82) resembling those found in the disease (83–86). And, reported reductions in CIV activity (87) and CI and CIV deficiencies in AD platelets and brain tissue (88–90) led to the idea that CIV deficiency could be behind AD pathogenesis (91, 92). However, detractors pointed out not only that the reported CIV reductions in AD are below the threshold for dysfunction (93, 94), but that similarly small reductions in CIV subunits were observed in other neurodegenerative diseases (95), implying that the CIV deficiencies likely reflected non–AD-specific changes. Moreover, as in PD, additional studies challenged the aforementioned findings, by showing that mitochondrial respiration capacity was unaffected in AD brain and arguing that metabolic alterations in AD are unlikely to be driven by primary OxPhos deficiencies (96). Finally, exposing cell cultures, isolated mitochondria, and cells to amyloid-β oligomers — key players in AD — resulted in mitochondrial dysfunction (97), thus reversing the purported cause and effect.

Can impaired OxPhos and low ATP induce neurodegeneration?

Before addressing the question of whether impaired OxPhos and low ATP can induce neurodegeneration, it is useful to summarize how mitochondrial energy is produced. From a broad view, oxidative energy metabolism comprises two elements: a respiratory chain that generates a proton gradient (derived from the NADH and $FADH_2$ that are produced in the Krebs cycle) across the inner mitochondrial membrane (IMM), and an ATP synthase that uses this gradient to power the conversion of ADP to ATP. The respiratory chain complexes (CI, CIII, and CIV, but not CII) embedded in the IMM pump these protons "vertically" from the matrix to the intermembrane space (IMS), while transferring the NADH- and $FADH_2$-derived electrons "horizontally" via a series of redox reactions: from CI and CII to coenzyme Q to CIII to cytochrome *c* to CIV and eventually to molecular oxygen (producing water) (Figure 1). The excess of H^+ ions in the IMS relative to their paucity in the matrix sets up a proton gradient favoring the movement of H^+ ions in the opposite direction, from the IMS to the matrix. These protons traverse the IMS through ATP synthase, which uses this "proton-motive force" to drive the conversion of ADP to ATP (98).

Defects in energy synthesis can arise at many steps in this process. For example, problems in glycolysis or the Krebs cycle can reduce the production of $NADH/FADH_2$; problems in proton pumping can reduce the strength of the gradient, reducing ATP synthesis efficiency; problems in electron flow can divert electrons from the respiratory chain to other molecules (e.g., proteins, lipids, DNA, and free oxygen [forming reactive oxygen species]); and alterations in the IMM's permeability can allow protons to dissipate across this membrane, bypassing ATP synthase (a phenomenon called "uncoupling"), and so forth.

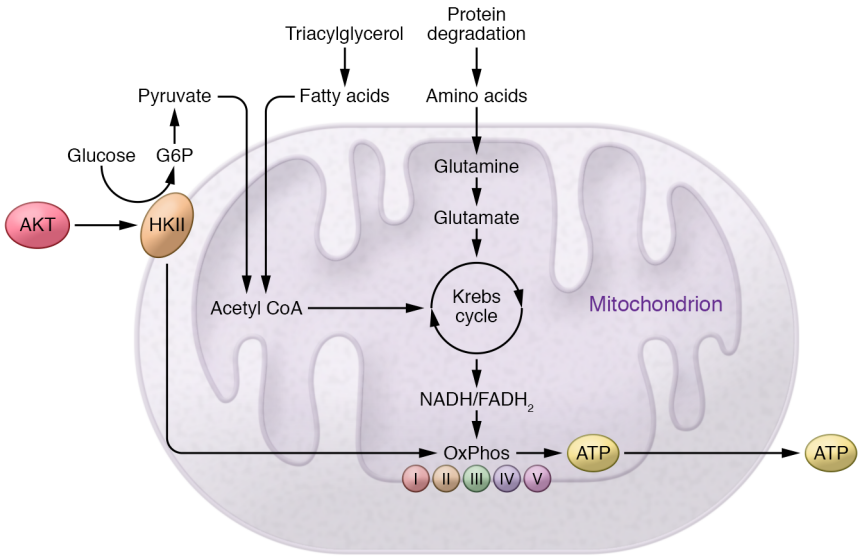

Figure 2. Mitochondrial metabolic network. Many metabolic pathways converge to maintain energy output. While most cells use glucose/pyruvate for ATP synthesis, oxidation of fatty acids and amino acids can also be used in response to changes in the cellular environment and availability of substrates. These mechanisms ultimately converge onto the Krebs cycle to produce NADH and $FADH_2$, which in turn feed the mitochondrial ETC. Possible mechanisms of OxPhos deficiency in neurodegenerative diseases involving the AKT/PKB pathway and pyruvate metabolism are shown. G6P, glucose 6-phosphate.

Moreover, mitochondria reside at the center of a complex network of metabolic pathways that can be modulated to counterbalance reductions in OxPhos to maintain cellular homeostasis. Thus, the correlation between an OxPhos defect and pathology is far from straightforward. Moreover, in any given cell, impaired mitochondria coexist with healthy mitochondria, which, above a certain threshold, will compensate for mitochondrial defects and permit a normal phenotype (99–101). This threshold, which is tissue-specific, depends on a number of factors (60). First, mitochondria, via changes in membrane lipid composition, can modulate the association of individual respiratory complexes into supercomplexes (102), thereby increasing the efficiency of ATP production, presumably by physically channeling redox substrates (e.g., coenzyme Q_{10} and cytochrome *c*), thereby mitigating the consequences of an OxPhos defect (103, 104).

Second, in most cell types mitochondria run at basal respiratory levels without using their total bioenergetic capacity (60). Only when a cell needs an additional surge of energy (e.g., because of increased activity or stress) do its mitochondria use their "reserve respiratory capacity" to increase substrate oxidation and/or ATP output (105). Thus, even in the presence of significant OxPhos defects, many cell types can avoid the consequences of reduced ATP by summoning this spare respiratory capacity. This plasticity may be the result of excess mt-mRNAs, mt-tRNAs, and/or a pool of respiratory complexes in "standby" (59) that act as a backup mechanism to compensate for OxPhos defects (60, 106).

Third, this reserve respiratory capacity can also be modulated by the different fuels feeding the Krebs cycle and OxPhos (60), in a tissue-dependent manner. For example, when faced with a general OxPhos defect, the brain can compensate so long as the diet is high in glucose (think grapes), because of its higher threshold for pyruvate utilization. However, a change to a diet high in fats and amino acids (think well-marbled steak) will not allow for such compensation, because of the brain's low threshold for nonpyruvate substrates such as succinate (58).

If reserve capacity can compensate for a defect in mitochondrial respiration to maintain a steady level of ATP, to what extent can OxPhos activity be reduced before the ATP content is low enough to trigger cell death? While reports vary, some groups found that, depending on the tissue, reductions of 70% to 85% for CI and CIII, and approximately 60% for CIV, were necessary to produce a significant decrease in ATP production (107–110). Thus, reported reductions of 30% to 50% in CI or CIV activities in PD and AD, respectively, would not necessarily impact on ATP production significantly.

In support of this idea of reserve capacity, a T8993G mutation in mtDNA-encoded *MTATP6*, specifying the ATP6 subunit of ATP synthetase (complex V [CV]), results in a 50%–80% reduction in mitochondrial ATP production (111), but not in overall ATP content, as long as glucose is available (112). This mutation provokes a clinical picture that is not reminiscent of any common neurodegenerative diseases, but rather of a syndrome characterized by neuropathy, ataxia, and retinitis pigmentosa (known as NARP; ref. 113). On the other hand, at high mutation loads (>90%) this same mutation can provoke a maternally inherited form of Leigh's syndrome (MILS) that can cause neuronal death in basal ganglia and midbrain (114, 115). Notably, the amount of ATP produced at the threshold for the NARP phenotype is only approximately 35% of the total ATP available (116, 117), implying that the remaining approximately 65% is still available for other non-OxPhos functions. Moreover, in these mitochondrial disorders, the loss of neurons observed within the affected brain areas follows a patchy, stochastic distribution (118, 119) rather than following a logic of differential susceptible subpopulations typical of neurodegenerative disorders. Indeed, in PD, nigral dopaminergic neurons die more than ventral tegmental area dopaminergic neurons (66); in ALS, motor neurons innervating fast fatiguing muscles die more than those innervating slow muscles (120); and in Huntington's disease, striatal neurons expressing GABA and enkephalin die more than those expressing GABA and substance P (121).

Finally, mitochondria are often analyzed as an isolated subcellular fraction. For that reason, it is quite common to extrapolate specific respiratory defects in those organelles to an overall bioenergetic defect in the cell or tissue from which the mitochondrial fraction was derived. However, as noted above,

mitochondria reside at the center of a complex metabolic network where multiple bidirectional mechanisms of adaptation are coregulated to maintain energy output (122). For example, the overall content of cellular ATP is the result of substrate-level phosphorylation of ADP via glycolysis in the cytoplasm and/or oxidative phosphorylation of ADP in the mitochondria. The latter is fueled mainly by the pyruvate produced during glycolysis, or to a lesser extent by fatty acid and/or amino acid oxidation (Figure 2). The rate and coupling of these pathways depend on cell type and are extremely adaptable to changes in energy demand and substrate availability (123). Under pathological conditions in which mitochondrial ATPase activity is reduced, most cells upregulate glycolysis and can completely bypass mitochondria to maintain sufficient levels of ATP (124). In fact, genetic or chemical inhibition of mitochondrial ATPase not only did not result in neurodegeneration, but actually prevented neuronal death by shifting ATP production from OxPhos to increased glycolysis (125, 126). In a second example, in ρ^0 cells (cells lacking mtDNA and hence OxPhos activity), glycolytically derived ATP generated in the cytosol can travel back into the mitochondria (112) to maintain other functions, such as calcium buffering capacity and lipid synthesis.

Overall, we can conclude that, clinically speaking, neurodegenerative disorders have little resemblance to primary mitochondrial disorders. Moreover, of all known mtDNA alterations in authentic mitochondrial disease, few have been proven to result in dementia, even in long-lived patients, and none have been unambiguously linked to the development of neurodegeneration.

Possible mechanism of OxPhos deficiency in neurodegenerative disease

Despite the controversies, we cannot exclude the fact that alterations in OxPhos occur in neurodegeneration. In the course of neurodegeneration, deficits in bioenergetics do often arise and could reduce the capacity of compromised neurons to withstand the actual disease process. However, we have espoused here the view that the OxPhos abnormalities documented in neurodegenerative disorders could be nonspecific features of dying cells. But even if this is the case, the question remains of how these mitochondrial alterations arise in the first place. Although several potential mechanisms may be proposed, including defects in mitochondrial autophagy, especially in the context of PD mutations in PINK1 and PARKIN, or in mitochondrial dynamics that have been reviewed elsewhere (127, 128), herein we would like to offer two other possible scenarios.

Alterations in pyruvate metabolism: the case of PD. Neuronal resilience to defects in glycolysis or OxPhos within a certain range is the consequence of the balanced cooperation between mitochondrial OxPhos and glycolysis (and to a lesser extent the pentose phosphate pathway) to maintain ATP levels. In the brain, metabolic flexibility depends not only on the interplay between glycolytic and OxPhos pathways and the nature of the fuel, but also on the communication between astrocytes and neurons (129). Briefly, astrocytic metabolism relies mostly on glycolysis to produce lactate to send to the neuron (129). In the neuron, lactate is converted into pyruvate (129), which then enters the mitochondria via the mitochondrial pyruvate carriers (130) to be converted into acetyl-CoA by pyruvate dehydrogenase (PDH) (131), feeding the Krebs cycle to power OxPhos.

Maintaining proper cell bioenergetics in these pathways requires coordination between glycolysis, lactate uptake, pyruvate entry into mitochondria, and OxPhos (131). One of the main orchestrators behind this coordinated effort is AKT, which, when activated, translocates to mitochondria, increasing the binding of the glycolytic enzyme hexokinase II (HKII) to the mitochondrial surface (132). The localization of HKII on mitochondrial membranes promotes both glycolysis and OxPhos (133), while activated AKT induces the expression of hexokinase and the glucose transporters (134, 135). Disruption of these pathways can alter glucose metabolism and pyruvate entry into mitochondria, reducing OxPhos (131, 136). Consequently, it is possible that during neurodegeneration, mitochondrial OxPhos deficit can be reinterpreted as a consequence of prior and sustained reductions in the production and transport of pyruvate to the mitochondria (137).

Interestingly, aberrant glucose and pyruvate metabolism has been described in AD and PD (138), and both disorders have been linked to type 2 diabetes (T2D) (139). Specifically, 60% of PD patients suffer various degrees of insulin resistance (140). Conversely, T2D patients have a 35% increased risk of developing PD (140–142) and a 65% increased risk of developing AD (143).

Early studies comparing metabolism in PD patients versus controls showed significantly lower preclinical glucose utilization (144) and reduced pyruvate oxidation in PD patient fibroblasts (145). In addition, significant elevations in pyruvate and decreases in succinate, malate, citrate, and acetate in PD patient blood suggest the presence of defects in PDH complex activity and in genes encoding PDH-interacting proteins (146). Similar reductions in glucose metabolism were also detected in animal models of PD (147–150) and in cells incubated with the neurotoxins MPTP or 6-hydroxydopamine (6-OHDA) (151). While the connections between glucose and PD had often been discussed as expected consequences of reduced CI activity, a hypothetical and early alteration in glucose metabolism would result in similar reductions in mitochondrial respiration (136).

Relevant to this possibility, dopaminergic neurons have been shown to be quite sensitive to hypoglycemic conditions, resulting in reduced tonic firing and membrane hyperpolarization (152). Moreover, in vitro glucose deprivation in neuronal cultures reduces dopaminergic uptake and promotes cytosolic aggregation of α-synuclein and dopaminergic neuron death (153). These data are also in support of reports showing that α-synuclein aggregation occurs prior to significant reductions in mitochondrial activity (154).

While the mechanism behind these alterations in glucose metabolism is unknown, the literature discusses several possibilities (155). For instance, AKT activation, a key regulator of glucose metabolism (134), has been shown to be significantly reduced in the substantia nigra of PD patients (156) and in cultured cells exposed to 6-OHDA (157). Furthermore, mutations in genes related to familial PD such as *DJ-1* or *PINK1* have also been linked to diminished AKT signaling (158). Remarkably, even some *AKT* (159) and *GSK3B* polymorphisms (160) are associated with a higher PD risk. Finally, another familial PD gene, *PARKIN*, is implicated in the regulation of the glycolysis-OxPhos connection (161) by binding

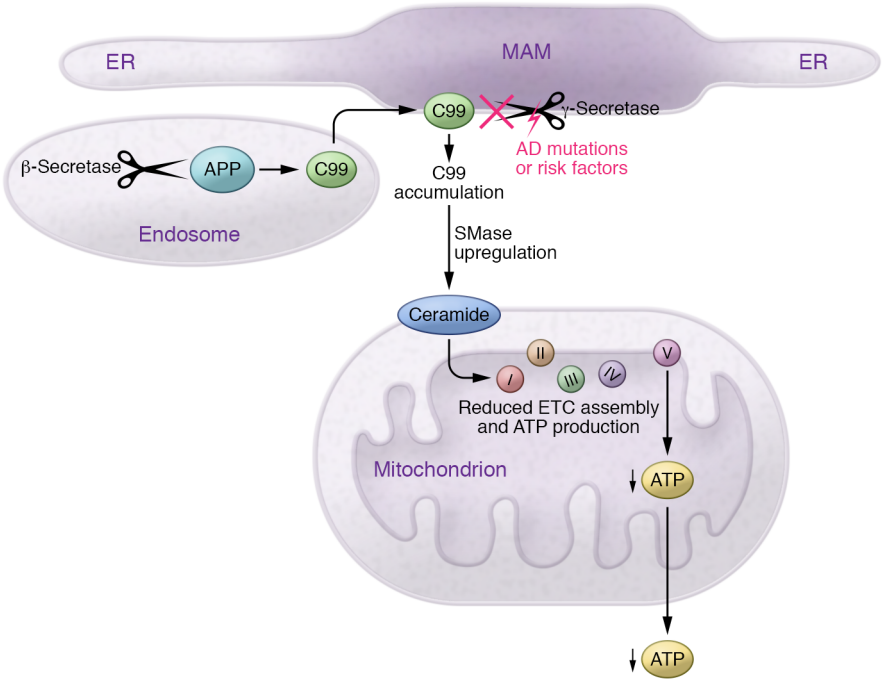

Figure 3. Mitochondria-associated membrane and AD. MAM is a specialized, lipid raft–like subdomain of the ER that communicates with mitochondria. APP is processed first by β-secretase in endosomes to produce C99. C99 then translocates to the ER (by an unknown mechanism), where it is cleaved rapidly by MAM-localized γ-secretase. In AD, however, this process is impaired, leading to the abnormal accumulation of C99 in the MAM, which correlates with increased conversion of sphingomyelin to ceramide via an upregulation of sphingomyelinase activity. In turn, the elevated ceramide compromises ETC assembly and ATP production.

and ubiquitylating pyruvate kinase M2 (PKM2) (162), decreasing its activity and glucose metabolic rate (131).

Alterations in ER-mitochondria contact sites: the case of AD. In the last decade, much evidence has been marshalled demonstrating that mitochondria are indeed "social organelles" by means of their membrane-to-membrane interaction with other organelles. Among these, ER domains that contact mitochondria, or mitochondria-associated ER membranes (MAMs; Figure 3), have been defined as functional platforms that contain a subset of enzymatic activities involved in regulating calcium, lipid homeostasis, mitochondrial dynamics, and a number of other functions previously ascribed to isolated mitochondria (163, 164). MAM domains form lipid raft–like structures that are regulated by their lipid milieu and by the proteins embedded within them. Thus, alterations in the conformation or subcellular localization of MAM-resident proteins or in MAM-associated lipid pathways can induce alterations in MAM-regulated functions and the crosstalk between ER and mitochondria. Of special relevance to our discussion is that many proteins related to neurodegeneration, especially in AD and PD, have been shown to translocate to MAM and participate in regulating these ER domains and their communication with mitochondria.

For example, we and others have shown that α-synuclein can translocate to MAM regions (165, 166), supporting the reported affinity of α-synuclein for lipid raft–like domains and its previously reported association with mitochondrial membranes. In addition, mutations in α-synuclein alter the activities localized in these domains, such as lipid metabolism (165) and calcium regulation (167).

In AD (Figure 3), presenilins, amyloid precursor protein, and γ-secretase activity were found to be enriched at MAM domains (168–170), which could explain previous reports suggesting a mitochondrial localization of these AD-related proteins (171, 172). Notably, as in PD, activities localized in MAM (including lipid homeostasis and calcium regulation) are altered in both familial and sporadic AD (173–175). These MAM-associated disturbances can impact mitochondrial biology by several mechanisms.

First, ER-mitochondria connections regulate calcium homeostasis, which is well known to be altered in AD (167, 176, 177). Among its many functions, mitochondrial calcium buffering capacity regulates the intracellular concentration of calcium, not only by buffering local changes in the cytosol, but also via highly regulated contacts with the plasma membrane and/or the ER (178, 179). Therefore, alterations in the communication between mitochondria and these organelles can significantly impact the entry of calcium into mitochondria, affecting overall calcium signaling in the cell (180, 181). Notably, calcium concentration is a regulator of rate-limiting enzymes in the Krebs cycle, and increases in calcium in the mitochondrial matrix activate the Krebs cycle and OxPhos (182). Thus, mitochondrial calcium buffering capacity not only balances intracellular calcium concentration, but also enables the coregulation of mitochondrial bioenergetics and ATP requirements in the cell. However, over a certain threshold, calcium increases in mitochondria can collapse membrane potential and induce the opening of the mitochondrial transition pore, triggering mitochondrial dysfunction and cell death (183). Taken together, these data imply that alterations in ER-mitochondria contact sites could initiate OxPhos defects through changes in calcium regulation, and likely play a significant role in AD pathogenesis.

Second, as mentioned above, MAMs are involved in the regulation of lipid homeostasis, including sphingolipid metabolism. Recent data indicate that brain tissues from AD models and AD patient–derived cells display significant increases in sphingomyelinase-mediated sphingomyelin hydrolysis within MAM domains (175). This increased sphingomyelinase activity reduced sphingomyelin content and elevated ceramide (the product of sphingomyelin hydrolysis) at ER-mitochondria contact sites and on mitochondrial membranes (175). Pharmacological reduc-

tion of ceramide levels in AD models and in AD patient cells rescued mitochondrial respiratory deficits (175), underscoring the detrimental effect of ceramides on OxPhos regulation (184, 185). These results illustrate how bioenergetic defects are plausible consequences of alterations in the regulation of sphingolipid turnover at MAM domains (185).

Several possibilities have been suggested to explain the mechanism behind sphingolipid-mediated disturbances in AD. Elevations in ceramide could alter mitochondrial membrane properties, hampering the conformation or assembly of respiratory complexes in the IMM (173) and negatively impacting respiratory rate (186). In addition, upon exceeding a threshold, ceramide can form channels in the outer mitochondrial membrane large enough to allow soluble proteins to translocate into the cytosol (187). These ceramide channels bind to, and are regulated by, pro- and antiapoptotic proteins, making this lipid an important player in the induction of apoptosis (187). We also note that increases in the local concentration of ceramide at the MAM "glue" mitochondria to tubulin via the outer membrane protein porin (also called the voltage-dependent anion channel [VDAC]) (155), which regulates mitochondrial transport, subcellular distribution, and division (188).

Third, one of the best-known activities localized to MAM regions is phospholipid synthesis and regulation (163). Alterations in MAM can affect the phospholipid milieu of mitochondrial membranes, including the signature mitochondrial lipid, cardiolipin (189, 190). Cardiolipin interacts with and regulates a number of mitochondrial proteins, including OxPhos complexes (191). In fact, this lipid is required for the proper assembly and functioning of all membrane-bound respiratory complexes and ATP synthase (192). Cardiolipin is also involved in the formation and stabilization of mitochondrial supercomplexes (193). Unsurprisingly, alterations in the concentration and/or transacylation of cardiolipin are associated with mitochondrial OxPhos defects (194) and with specific mitochondrial disorders, such as Barth syndrome (195, 196). Cardiolipin could also influence bioenergetics indirectly, including regulation of the orientation of the ADP/ATP carrier in the IMM (197) via modulation of mitochondrial creatine kinase activity (198), or even via regulation of BAX oligomeric pores on mitochondrial outer membrane (199).

Concluding remarks

In the past three decades, tremendous progress has been achieved in our understanding of mitochondrial biology in health and disease. Deciphering the mitochondrial genome provided insights into the catalog of genetic mutations linked to primary mitochondrial diseases and to the observation that the same genetic mutation may produce a similar defect, i.e., OxPhos deficiency, and yet the clinical presentation may be varied. This realization prompted investigators to embark on studies based on the following: if mitochondrial mutations (using OxPhos deficiency as a proxy) can give rise to different neurological syndromes that had no known etiology, then other neurological disorders could actually be unsuspected mitochondrial diseases. Almost serendipitously, PD and AD emerged as perfect candidates for the concept of a mitochondrial etiology, followed by other neurodegenerative disorders. In many instances, while studies found no consistent genetic basis, they documented alterations of OxPhos activity in patients as well as in animals and cellular models of human neurodegenerations.

We have argued in this Review that many of these findings, while appealing, are generally correlative and fail to demonstrate causality. We have also contended that the interpretation of OxPhos defects in neurodegeneration too often stems from studies of isolated mitochondria. These facts did not deter many investigators from regarding neurodegenerative disorders as atypical forms of mitochondrial diseases. Admittedly, even if such a view may not be accurate, and it turns out that OxPhos alterations are mere consequences of initial insults that trigger the disease, a deficit in bioenergetics can still contribute to the pathogenesis and be a valuable therapeutic target. Thus, it will be important to pursue investigations aimed at elucidating how bioenergetics can be improved. Investigations should also be encouraged to elucidate the origin of an OxPhos defect in neurodegeneration, even if it is a secondary alteration, because the most effective therapy for neurodegenerations such as AD or PD is likely going to be mechanism-specific. As such, we have proposed herein two "out-of-the-box" mechanisms in an attempt to broaden the conversation about how OxPhos arises in, and contributes to, neurodegeneration. Indeed, the lack of answers after more than 30 years and dozens of published papers should make us wonder whether the clues mentioned in the introduction are misleading us and whether it is time to rethink the search.

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