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The role of cytochrome *c* oxidase deficiency in ROS and amyloid plaque formation

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Abstract The multiple dysfunctional changes associated with a brain affected with Alzheimer's disease (AD) makes the understanding of primary pathogenic mechanisms challenging. Mitochondrial dysfunction has been associated with almost every neurodegenerative disease and neurodegenerative-related event. Alzheimer's disease is no exception with data suggesting mitochondrial malfunctions ranging from improper organelle dynamics, defective oxidative phosphorylation (OXPHOS), oxidative stress, and harmful beta amyloid (Aβ) associations with the mitochondria. A major change often associated with AD is impairment of the electron transport chain at complex IV: cytochrome *c* oxidase (COX). This mini-review concentrates on recent work by our group that sheds light on the role COX deficiency plays in the pathophysiology of AD using a transgenic mouse model. Results suggest that neuronal COX deficiency does not increase oxidative stress and nor increases amyloidal formations *in vivo.* Conclusions from this work also suggest that Aβ formation is a cause of COX deficiency as opposed to the consequence.

Keywords Alzheimer's disease · COX deficiency · Amyloid formation · Oxidative stress

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Introduction

Alzheimer's disease is an age-related neurodegenerative disorder accompanied by the progressive loss of cortical and hippocampal neural populations and concomitant dementia with declines in cognition until death. Major pathological features of AD include the accumulation of Aβ deposits and appearance of intracellular tangles composed of hyperphosphorylated tau.

According to the 'amyloid hypothesis', the major cause of dysfunction arises from the presence of harmful $A\beta_{1-42}$ species that accumulate as intraneuronal and extracellular species (reviewed in (Lansbury and Lashuel 2006)). Which conformational form of this protein, as monomers, oligomers, or aggregates, causes this synaptic dysfunction and neurodegeneration is still under debate. However, the noxious presence of Aβ *in vitro* and *in vivo* has continually held up over the years regardless of whether the pathology of AD has been completely, faithfully modeled (Loo et al. 1993; Morgan et al. 2000; Rajendran et al. 2008).

There is a strong relationship between Aβ and the mitochondria. Aβ has been shown to directly associate with and penetrate mitochondria as intraneuronal soluble proteins before depositing as insoluble, extracellular species (Manczak et al. 2006). Inside the mitochondria, Aβ has been showed to associate with Aβ alcohol dehydrogenase (ABAD) to directly inhibit mitochondrial function, increase reactive oxygen species (ROS), and release cytochrome *c* to

initiate intrinsic apoptotic pathways (Lustbader et al. 2004). Aβ interactions with cyclophilin D, a suggested subunit of the permeability transition pore, may also contribute to similar mitochondrial dysfunctions and exacerbate cell death (Du et al. 2008). Aβ has also been shown to disrupt normal mitochondrial dynamic processes and morphology leading to its fragmentation (Wang et al. 2008; Cho et al. 2009). These studies are only a few of the many examples citing the different ways Aβ affects and disrupts normal mitochondrial function.

With all of these individual events affecting mitochondrial function, it is surprising that only one component of the electron transport chain, COX, has been consistently found to be defective in the affected tissues (Mutisya et al. 1994; Chagnon et al. 1995). This biochemical impairment is accompanied by increased oxidative damage, which has been evaluated by the levels of oxidative modifications of macromolecules (Lustbader et al. 2004; Devi et al. 2006; Manczak et al. 2006). One plausible explanation for this observation would be the direct or indirect toxic effect of Aβ on COX and the production of ROS. Indeed, a series of *in vitro* experiments supports this possibility (Casley et al. 2002; Rhein et al. 2009; Xu et al. 2009). But what explains the age-dependent accumulation of Aβ in the first place?

We thought of the possibility that mitochondrial abnormalities could be the cause of Aβ accumulation. Aging is a strong risk factor for AD, and aging itself is manifested by the decline of mitochondrial oxidative phosphorylation and elevation of oxidative stress. One hypothesis was that individuals who are more affected by age-associated mitochondrial abnormalities in a stochastic fashion have a higher risk for developing sporadic AD. In this scenario, the deficit in mitochondrial oxidative phosphorylation promotes the accumulation of Aβ, leading to the diseased state. To explore this possibility, we have evaluated Aβ accumulation in the context of neuron-specific COX deficiency *in vivo*. Surprisingly, we found that COX deficiency reduced oxidative stress and depressed amyloid formation. These results suggest that COX deficiency originates from the presence of $A\beta_{1-42}$ as opposed to facilitating the production of Aβ or the formation of amyloid plaques. Also, these results suggest that Aβ may enhance ROS formation through mechanisms other than through mitochondrial COX deficiency.

COX-deficient neurons have reduced oxidative stress and amyloid formation

Our laboratory has created mouse models with defective respiratory complexes to better understand aspects of neuromuscular and central nervous system cellular susceptibilities. From these projects, we created a mouse model with a conditional genetic disruption of the *COX10* gene (Diaz et al. 2005). This *COX10* genetic disruption causes the incomplete assembly of COX and no longer produces functional subunit COXI (Diaz et al. 2006). Crossing this animal with another line expressing *CaMKIIα-Cre* recombinase produced a COX deficiency in cortical and hippocampal neurons (Dragatsis and Zeitlin 2000; Jankowsky et al. 2001). These mice were further bred with an AD transgenic mouse model expressing both mutant amyloid precursor protein (APP) and mutant presenilin 1 (PSEN1). The final progeny resulted in an *in vivo* model of neuronal COX deficiency in which we could study the consequences of COX deficiency in the AD diseased state. This triple transgenic approach allowed for the first time a model to study the interaction between the coinciding events of COX dysfunction and Aβ accumulation.

Plaque burden is reduced in AD transgenic animals with conditional neuronal COX deficiency

Stereological immunohistochemical identification of Aβ-positive plaques of both dense and diffuse morphology showed significantly lower amounts of plaques in COX-deficient AD brains as compared to COX-competent AD controls in the hippocampus and cortex (Fukui et al. 2007). Furthermore, a colorimetric ELISA assay confirmed that the total level of $A\beta_{1-42}$ was significantly reduced in the forebrains of AD animals with the COX deficient background as compared to the AD controls (Fukui et al. 2007). These results suggest that COX deficiency negatively affects Aβ pathology.

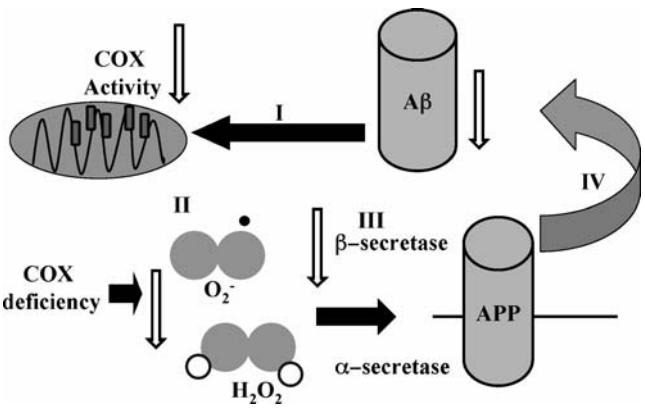

Fig. 1 Summary model of the effects of COX deficiencies in AD pathology. White outlined arrows depict events that were significantly attenuated by COX deficiency. I. $A\beta_{1\_42}$ affects mitochondrial function leading to COX deficiency. II. Reduction in ROS leads to subsequent curtailment of both β-secretase activity (III) and $A\beta_{1\_42}$ production and formation of Aβ immunopositive species (IV)

COX deficiency reduces oxidative damage and β-secretase activity

Examination of *in vivo* markers of oxidative stress showed a reduction in protein carbonyl modifications and a reduction in immunoreactive 8-hydroxy-2′-deoxyguanosine (8-OH-dG) nucleic acids in AD COX deficient animals compared to AD controls (Fukui et al. 2007). These results suggest that oxidative stress present in AD does not arise from dysfunctional COX activity. Considering the previous work showing the modulation of β-secretase activity by oxidative stress in cultured neuronal cells (Paola et al. 2000; Tamagno et al. 2002), we hypothesized that the activity of β-secretase may be affected in the COX-deficient brains. The measurement of β-secretase activity using a fluorogenic substrate in the cortical lysates showed that β-secretase activity was actually reduced in the COX-deficient AD animals. Therefore, reduced β-secretase activity might underlie the reduced production of $A\beta_{1-42}$ in our conditional KO model.

Aβ promotes COX dysfunction in AD

The activity of COX was reduced in the cerebral cortices of APP/PSEN1 transgenic mice, suggesting that increased Aβ production inhibits COX activity (Fukui et al. 2007). Our result confirms observations also made by others in cell culture and *in vitro* models (Casley et al. 2002; Anandatheerthavarada et al. 2003). The work of other groups also has tried to elucidate the molecular mechanisms underlying this COX defect. Data suggest Aβ directly binds with heme-α, an essential component of COX. This sequestration of heme-α by Aβ then leads to the decrease of COX activity (Atamna and Boyle 2006). Another study associated amyloid precursor protein with the import channels causing blockage for incoming COX subunits IV and Vb as potentially a source causing the decrease in COX activity (Devi et al. 2006). Also, Aβ associated with Aβ alcohol dehydrogenase seemed to depress COX activity, but an explanation for this result has not been offered (Lustbader et al. 2004). Although these studies led to a better understanding of the biochemical changes in AD, more research is still needed to continue to elucidate this mechanism.

Future directions to further elucidate contributions of oxidative phosphorylation deficiencies to Alzheimer's disease pathologies

We obtained unexpected results showing that a COX deficiency actually reduces Aβ formation, oxidative stress and plaque formation (Fig. 1). This goes against the wide believe that OXPHOS dysfunction stemming from the reduction of COX would lead to increased oxidative damage. Clearly, more work is necessary to understand the relationship between OXPHOS, ROS and AD.

The biggest risk factor for individuals to develop sporadic AD is aging. The expression of nuclear genes encoding components of oxidative phosphorylation and other mitochondrial proteins declines with age (Hong et al. 2008). Nuclear DNA is not the only genome to sustain significant changes with age. Mitochondrial DNA (mtDNA) has also been shown to undergo age-related mutational changes in healthy people and those suffering from neurodegenerative diseases, subsequently leading to decreased components of OXPHOS (Corral-Debrinski et al. 1992; Lin et al. 2002; Bender et al. 2006). It has also been reported that AD patients harbor more somatic point mutations in the control region of their mtDNA leading to reduced transcripts and mtDNA copy number (Coskun et al. 2004). In addition, base pair changes in this particular region have direct consequences on the binding of mitochondrial proteins to the genome thus affecting maintenance, replication, transcription, and translation (Suissa et al. 2009). These nuclear and mitochondrial DNA changes occurring with age would reduce global oxidative phosphorylation and general energy metabolism. Moreover, other OXPHOS defects may contribute to ROS formation more significantly than isolated COX deficiencies.

Currently, we are moving forward with other genetically manipulated mouse models that have reduced levels of other OXPHOS complexes to understand if age-dependent decreases in bioenergetic function differently affect the accumulation of Aβ.

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