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Alterations in mitochondrial number and function in Alzheimer's disease fibroblasts

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Abstract

Introduction—Mitochondrial dysfunction is observed in brains of Alzheimer's Disease patients as well as many rodent model systems including those modeling mutations in presequence 1 (PSEN1). The aim of our study was to characterize mitochondrial function and number in fibroblasts from AD patients with PSEN1 mutations.

Methods—We used biochemical assays, metabolic profiling and fluorescent labeling to assess mitochondrial number and function in fibroblasts from three AD patients compared to fibroblasts from three controls.

Results—The mutant AD fibroblasts showed increased AB42 relative to controls. Reductions in ATP as well as basal and maximal mitochondrial respiration were also observed in these fibroblasts as was impaired spare mitochondrial respiratory capacity. Fluorescent staining and expression of genes encoding electron transport chain enzymes showed diminished mitochondrial content in the AD fibroblasts.

Conclusions—This study demonstrates that mitochondrial dysfunction is observable in AD fibroblasts and provides evidence that this model system could be useful as a tool to screen disease-modifying compounds.

Keywords

β -amyloid; mitochondrial dysfunction; Alzheimer's disease

Introduction

While genetic forms of Alzheimer's disease (AD) do not account for the majority of cases, there are several known mutations that result in the disease. Mutations in presenilin-1 (PSEN1) result in the most prevalent form of inherited AD (Bekris 2010, Elder 2010). PSEN1 is a key component of the γ -secretase complex which mediates one of the cleavage events that converts amyloid precursor protein into A β . Mutations in PSEN1 increase the production of A β 42 which is more likely to oligomerize and accumulate. This

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accumulation of A β is associated with mitochondrial dysfunction and cognitive impairment (Du 2010, Rhein, et al. 2009, Yao 2009). In vitro studies suggest that a relationship between A β accumulation and mitochondrial function (Hensley 1994, Rhein 2009). The hypothesis that mitochondrial dysfunction contributes to cognitive decline has led to a growing interest in identifying therapies that target mitochondrial function as a treatment for AD. Yet physiologically relevant model systems to test such treatments remain limited.

The aim of this was to characterize mitochondrial function in human fibroblasts to determine whether abnormalities are observable in a defined population of AD patients. A variety of biochemical techniques were used to compare mitochondrial number and content of fibroblasts from AD patients with mutations in PSEN1 to control fibroblasts.

Methods

Cell Culture Fibroblasts

Fibroblasts were obtained from 3 AD patient with PSEN1 mutations, and 3 healthy controls (NINDS repository – Coriell) and cultured in growth media containing MEM α (Gibco) with 10%FBS and 1% penicillin-streptomycin (Sigma-Aldrich).

ATP Quantification

ATP was quantified using ATP determination kit (Invitrogen) as per the manufacturer's instructions and normalized to protein content determined by bicinchoninic acid (BCA).

Analysis of Mitochondrial Function

Mitochondrial function was assessed using the Seahorse Bioscience XF24 Extracellular Flux Analyzer. Fibroblasts were plated in growth medium at a density of in 150,000 cells/well, which was determined to be optimal for basal O₂ consumption rate (OCR). The following day cells were rinsed in assay medium (pH 7.4) containing XF Base medium (Seahorse Bioscience), 5.5mM glucose and 1mM sodium-pyruvate. Cells remained in assay medium 1h at 37 C in a non-CO₂ incubator prior to initializing the Seahorse24XF analysis. Using the MitoStress Kit as previously described (Wu 2007), OCR was measured under basal conditions as well as after sequential treatment with the ATP synthase inhibitor oligomycin (1 μ M), an ETC accelerator, *p*-trifluoromethoxy carbonyl cyanide phenyl hydrazone (FCCP at 1.5 μ M and the mitochondrial inhibitors rotenone (1 μ M) and antimycin (1 μ M). Three measurements were taken under each condition. The respiration following antimycin and rotenone treatment was used to correct for non-mitochondrial oxygen consumption. Data was normalized to total DNA content, which was determined from each well using the CyQuant kit (Invitrogen) as per the manufacturer's instructions.

Quantitative Real Time PCR

Cells were harvested and RNA was extracted using Tri-Reagent (Molecular Research Center). RNA was reverse transcribed with the Superscript III First Strand Synthesis kit (Invitrogen) as per the manufacturer's instructions. Relative gene expression was determined using TaqMan Gene Expression Master Mix (Invitrogen) and commercially available TaqMan primers (Invitrogen) for mitochondrially encoded NADH dehydrogenase 1 (Mt-

ND1), mitochondrially encoded ATP synthase 6 (Mt-ATP6), mitochondrially encoded cytochrome c oxidase 1 (Mt-CO1), mitochondrially encoded cytochrome B (Mt-CYB) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Quantitative PCR (qPCR) was performed on a StepOne Plus Machine (Applied Biosystems) and analyzed using the delta-delta Ct method.

Mitochondrial Number

Relative mitochondrial number was determined using MitoTracker Green dye (Invitrogen) as per the manufacturer's instructions. Briefly cells were incubated with 100nM dye for 25 minutes, rinsed in fresh media and fluorescence was quantified using a Victor 3 fluorescent plate reader (Perkin Elmer).

Statistics

Statistical significance was determined using t-tests. Bonferroni post-hoc tests were also conducted. Significance was defined as $p < 0.05$. Analyses were performed using Excel or GraphPad Prism 6.

Results

Mitochondrial function is impaired in AD fibroblasts

ATP levels were significantly reduced in AD fibroblasts (Figure 1A). The metabolic profile of mitochondrial function revealed concordant deficiencies in cellular and mitochondrial oxygen consumption in the AD fibroblasts (Figure 1B). After adjusting for non-mitochondrial respiration both basal and maximal respiration were decreased in AD fibroblasts as was spare capacity (Figure 1C).

Mitochondrial Content is decreased in AD fibroblasts

Decreased mitochondrial number was also observed in AD fibroblasts as evidenced by reduced MitoTracker fluorescence (Figure 2D). Consistent with this finding, decreased expression of several electron transport chain (ETC) genes was also seen in AD fibroblasts relative to controls (Figure 2E). Expression of genes encoding enzymes in complexes I, III, IV and V were all reduced by approximately 50% in AD fibroblasts.

Discussion

Neuronal mitochondrial dysfunction is an early and prominent feature of AD (Wang 2009) as is reduced energy metabolism in the AD brain (Landau 2011, Shokouhi 2013, Yamane 2013). Diminished neuronal expression of genes encoding subunits of the ETC, as well as decreased expression or activity of many enzymes involved in oxidative metabolism, are also well-documented in AD brains (Cottrell 2001, Gibson 1998, Nagy 1999, Parker 1994). Calcium dyshomeostasis, altered glucose metabolism, and increased oxidative stress have all been observed in peripheral tissue including fibroblasts (Peterson 1986, Sims 1987, Torres 2011). In this study we have demonstrated that in a genetically defined population of AD patients, alterations in mitochondrial number and bioenergetic profile are indeed observable outside of the brain.

Fibroblasts from AD patients with PSEN1 mutations were shown to have decreased ATP content and significant impairments in mitochondrial respiration. Decreased basal and maximal respiration, as well as diminished spare capacity were observed in AD fibroblasts. Consistent with the decrease in basal respiration, genes encoding enzymes in the ETC were also found to be coordinately down-regulated in AD fibroblasts indicative of a reduction in mitochondrial number which was similarly observed using fluorescent mitochondrial labeling. Mitochondrial gene expression was decreased by approximately 50% in the AD fibroblasts while the fluorescent labeling showed a decrease of only about 25% in those fibroblasts. This discrepancy is likely due to differences in sensitivity of the assays as qPCR is a far more sensitive technique than quantifying fluorescence.

The findings from this study are in line with previous reports of mitochondrial changes in the brain of AD patients as well as in animal models of AD. Altered brain mitochondrial function can be detected by NMR in AD mice (Lalande 2014, Lin 2014) as well as human AD patients (Targosz-Gajniak 2013, Zhong 2014)(Fayed 2011). The cause of this mitochondrial dysfunction has yet to be elucidated, although increased AB accumulation has been implicated. A β treatment of cultured neuronal-derived cells results in ROS generation, decreased ATP production, and disrupted mitochondrial membrane potential (Hensley 1994, Rhein 2009) and synaptic A β accumulation in primary neurons leads to mitochondrial dysfunction and synaptic degradation (Du 2010). As has been previously reported (Johnston 1994, Sproul 2014) we also observed greater AB42 accumulation in the AD fibroblasts as compared to controls (data not shown). Yet the extent to which this AB accumulation contributes to the mitochondrial abnormalities observed in AD fibroblasts remains to be seen.

The consistent association between mitochondrial dysfunction and AD has led to the hypothesis that these mitochondrial changes precede and may contribute to the cognitive decline in AD (Leuner 2012, Swerdlow 2007). The fact that these changes are also observable in fibroblasts suggests potential utility of this as a model system as a physiologically relevant and more accessible method to screen for disease-modifying compounds. Future experiments are needed with fibroblasts isolated from idiopathic AD to validate this system beyond genetic AD patients.

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A. **B.** **C.** **Figure 1. AD fibroblasts have impaired mitochondrial function**

A) AD fibroblasts had significantly lower levels of intracellular ATP than control fibroblasts (n=8–12 replicates for each of 3 AD or control cell lines). B) AD fibroblasts displayed significantly reduced cellular and mitochondrial oxygen consumption rate (OCR) as compared to control fibroblasts under all assay conditions (n=4–6 for each of 3 AD or control cell lines). C) After correction for non-mitochondrial oxygen consumption, AD fibroblasts had diminished basal and maximal mitochondrial respiration. (n=4–6 for each of 3 AD or control cell lines). *p<0.05, **p<0.01 ***p<0.001.

Figure 2. AD fibroblasts have reduced mitochondrial content

A) AD fibroblasts showed a significant reduction in mitochondrial labeling by the fluorescent dye MitoTracker Green relative to control fibroblasts (n=8 for each of 3 AD or control cell lines). B) Expression of genes that encode several ETC enzymes was coordinately reduced in AD fibroblasts relative to controls (n=8–10 for each of 3 AD or control cell lines). *p<0.05, ***p<0.001.