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Altered age-related changes in bioenergetic properties and mitochondrial morphology in fibroblasts from sporadic amyotrophic lateral sclerosis patients

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ABSTRACT

Mitochondria play a key role in aging, which is a well-established risk factor in amyotrophic lateral sclerosis (ALS). We have previously modeled metabolic dysregulation in ALS using fibroblasts isolated from sporadic ALS (SALS) and familial ALS patients. In the present study, we show that fibroblasts from SALS patients have an altered metabolic response to aging. Control fibroblasts demonstrated increased mitochondrial network complexity and spare respiratory capacity with age which was not seen in the SALS cases. SALS cases displayed an increase in uncoupled mitochondrial respiration, which was not evident in control cases. Unlike SALS cases, controls showed a decrease in glycolysis and an increase in the oxygen consumption rate/extracellular acidification rate ratio, indicating an increased reliance on mitochondrial function. Switching to a more oxidative state by removing glucose with in the culture media resulted in a loss of the mitochondrial interconnectivity and spare respiratory capacity increases observed in controls grown in glucose. Glucose removal also led to an age-independent increase in glycolysis in the SALS cases. This study is, to the best our knowledge, the first to assess the effect of aging on both mitochondrial and glycolytic function simultaneously in intact human fibroblasts and demonstrates that the SALS disease state shifts the cellular metabolic response to aging to a more glycolytic state compared with age-matched control fibroblasts. This work highlights that ALS alters the metabolic equilibrium even in peripheral tissues outside the central nervous system. Elucidating at a molecular level how this occurs and at what stage in the disease process is crucial to understanding why ALS affects cellular energy metabolism and how the disease alters the natural cellular response to aging.

1. Introduction

Evidence suggests that mitochondria play an important role in aging, a process which is a crucial risk factor for neurodegenerative disorders (Ahmed et al., 2007; Karni-Schmidt et al., 2007; Tran et al., 2007). Mitochondrial metabolic dysfunction, the buildup of reactive oxygen species (ROS), altered calcium buffering and mutation of mitochondrial DNA (mtDNA) have all been observed with aging (Ahmed et al., 2007; Lin and Beal, 2006; Reddy, 2008; Swerdlow, 2007). The free radical theory of aging suggests that increased ROS production with age decreases mitochondrial respiration through damage to the respiratory chain complex (Harman, 1956; Kirkwood, 2005), which could lead to the production of more ROS in a vicious cycle (Indo et al., 2007). However, it is unclear whether ROS are the main cause of aging or the result of increasing mitochondrial dysfunction (Hekimi et al., 2011). It has been hypothesized that a decrease in the mitochondrial respiratory reserve capacity (adenosine triphosphate [ATP] production produced by oxidative phosphorylation at maximal compared to basal respiration) contributes to aging (Desler et al., 2012). A common factor in sporadic neurodegenerative diseases is the gradual deterioration in the function of the respiratory chain complexes (Lin and Beal, 2006). Oxidative phosphorylation and mitochondrial function in general are crucial for the ability of the nervous system to withstand cellular insults. These functions are especially important in the brain, which weighs 2% of the total body weight but consumes 20% of the total energy (Attwell and Laughlin, 2001).

In amyotrophic lateral sclerosis (ALS), it has been established across multiple animal and cellular models that mitochondrial dysfunction plays a key role in disease pathogenesis. The presence of ALS leads to defects in mitochondrial morphology, calcium

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buffering, the electron transport chain, ATP production, membrane potential, and axonal transport (Borthwick et al., 1999; Bowling et al., 1993; Carri et al., 1997; De Vos et al., 2007; Ferraiuolo et al., 2011; Fujita et al., 1996; Mattiazzi et al., 2002; Menzies et al., 2002). A clear relationship has also been established between mitochondrial metabolic dysfunction and altered mitochondrial morphology (De Vos et al., 2005; Shutt et al., 2012). Accordingly, fragmentation of the mitochondrial network, alongside swelling of mitochondrial cristae, has previously been observed in the post-mortem central nervous system (CNS) of individuals with sporadic ALS (SALS) (Sasaki and Iwata, 2007). This observation has been supported in animal models, where motor neurons derived from G93A mSOD1 transgenic mice displayed a decreased mitochondrial aspect ratio, suggestive of a "rounding up" of individual mitochondria (De Vos et al., 2007). Additionally, in cellular models of ALS expressing mutant SOD1, fragmentation of the mitochondrial network, decreases in mitochondrial length as well as significant remodeling of the mitochondrial cristae have been observed (Magrane et al., 2009; Menzies et al., 2002; Raimondi et al., 2006).

Peripheral tissues such as fibroblasts have been used to model neurodegenerative disorders as they replicate several pathophysiological abnormalities present in the affected tissues. For example, metabolic dysfunction, oxidative stress, and altered mitochondrial morphology have been observed in fibroblasts cultured from individuals with Alzheimer's disease, Parkinson's disease, and ALS (Mead et al., 2013; Mortiboys et al., 2010; Ramamoorthy et al., 2012). Recent work from our laboratory has shown that fibroblasts isolated from ALS patients with a SOD1 mutation display defects in mitochondrial function, which lead to a decrease in ATP production by the mitochondria. However, these cells are able to upregulate glycolysis as a compensatory response to the energy deficit (Allen et al., 2014). Mitochondrial respiration defects were also observed in fibroblasts isolated from SALS patients (Raman et al., 2014). It was unclear whether glycolysis was upregulated in this SALS model, as we observed age-dependent changes in glycolytic flux.

To date, the impact of age on mitochondria in ALS has not been formally investigated. Therefore, to determine the impact of age on mitochondrial bioenergetics and cellular metabolic function in SALS patient fibroblasts, we have used an XF24 bioanalyser (Seahorse Bioscience). The XF24 bioanalyser allows noninvasive, real-time analysis of metabolic parameters and mitochondrial functions, within the physiological environment of the cell. We have also investigated mitochondrial morphology and ATP production under the same metabolic conditions.

2. Methods

2.1. Human fibroblast biosamples

Experiments were carried out using fibroblasts obtained from a total of 6 SALS cases and 10 age- and sex-matched controls (see Table 1). The average age at the time of skin biopsy in ALS cases and controls was 64 years (range 39–78 years) and 57 years (range 37–77 years), respectively. In the ALS cases, the average age of disease onset was 62 years, with average disease duration of 37.9 months from date of symptom-onset to date of death. The SOD1 cases used in this study had an average age at time of skin biopsy of 55.33 years (3 unrelated male cases with an I113T mutation, range 44–62 years), and 53.66 years (range 38–63 years) for the six age- and sex-matched controls. The metabolic assays were originally performed as a separate group to the sporadic cases in a previously published study (Allen et al., 2014). For the purpose of this study, we have reanalyzed the SOD1 data focusing on the metabolic equilibrium measurements analyzed for the sporadic cases to compare directly to the SOD1 cases.

2.2. Fibroblast cultures

Skin biopsies were obtained from the forearm of subjects under sterile conditions and after informed consent, in accordance with guidelines set by the local ethics committee. Fibroblast cell cultures were established at the Metabolic Biochemistry and Tissue Culture Unit of the Sheffield Children's NHS Foundation Trust. Monolayers of primary fibroblast cell cultures were routinely maintained in T75 flasks in fibroblast cell culture medium (FCCM-PAA) minimal essential media, with 1 mg/mL glucose (PAA E15-825) supplemented with 10% FCS gold (PAA A15-151), 2-mM glutamine (Lonza BE17-605E), 50-μg/mL uridine (Sigma U3003), vitamins (Lonza 13–607C 1/100 dilution), amino acids (Lonza BE13-114E 1/100 dilution), 1-mM sodium pyruvate (Lonza BE13-115E), and 1-U/mL penicillin/1-μg/mL streptomycin (Lonza BE17-603E) in humid incubators at 37 °C supplemented with 5% CO_2 unless stated otherwise. For all assays, patient lines were matched to control lines not only in terms of sex and age but also in passage number to overcome any passage-dependent effects on mitochondrial morphology, respiration, and glycolytic flux. All assays were performed at passages 8–10.

2.3. Mitochondrial morphology assays

The fibroblasts were plated at a density of 32,000 cells per well, in a 6-well plate (Greiner Bio-One, Germany), onto 22 × 22 mm coverslips (Menzel Gläser, Germany). After 24 hours culture in FCCM, supplemented with either 1-mg/mL D-glucose (to promote glycolytic energy production) or in the absence of glucose and the presence of 0.01-mg/mL D-galactose (to promote mitochondrial energy production via the tricyclic antidepressant cycle), the cells were incubated for 3 minutes at 37 °C in 132-nM CMXRos MitoTracker Red (Invitrogen, USA) in conditioned media. After incubation, the cells were washed 3 times in FCCM for 1-minute intervals. The coverslips were then mounted onto a 76 × 26 mm microscope slide (Thermo Scientific, USA), in 200-μL conditioned glucose or galactose FCCM using high vacuum grease. Coverslips were sealed using nontoxic sealant grease composed of equal parts lanolin, vaseline, and paraffin wax. All imaging was carried out at ambient temperature (20 °C–21 °C) on a Zeiss Axioplan 2 fluorescent light microscope using a 63× Plan Apochromat 1.4 oil immersion objective. Images were optimized by Openlab5 software (PerkinElmer) and captured by a charge-coupled device (CCD) camera C4880-80 (Hamamatsu, Hertfordshire). All image analysis was carried out using ImageJ software (by W. Rasband,

Table 1
Summary of the fibroblasts used in the present study

Fibroblast ID	Age at date of biopsy (y)	Gender	Clinical diagnosis	Age at onset of symptoms	Disease duration (mo)
FIBCON02	44	Female	Control	—	—
FIBCON03	60	Female	Control	—	—
FIBCON05	54	Female	Control	—	—
FIBCON08	37	Female	Control	—	—
FIBCON11	54	Male	Control	—	—
FIBCON13	53	Female	Control	—	—
FIBCON14	41	Male	Control	—	—
FIBCON19	77	Female	Control	—	—
FIBCON156	74	Female	Control	—	—
FIBCON157	76	Female	Control	—	—
FIBPAT18	54	Male	sALS	53	47
FIBPAT21	67	Female	sALS	66	16
FIBPAT23	71	Female	sALS	70	28
FIBPAT26	39	Male	sALS	38	32.5
FIBPAT27	75	Male	sALS	71	54
FIBPAT31	78	Female	sALS	74	50