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Disruption of skeletal muscle mitochondrial network genes and miRNAs in amyotrophic lateral sclerosis

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ABSTRACT

Skeletal muscle mitochondrial dysfunction is believed to play a role in the progression and severity of amyotrophic lateral sclerosis (ALS). The regulation of transcriptional co-activators involved in mitochondrial biogenesis and function in ALS is not well known. When compared with healthy control subjects, patients with ALS, but not neurogenic disease (ND), had lower levels of skeletal muscle peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) mRNA and protein and estrogen-related receptor-α (ERRα) and mitofusin-2 (Mfn2) mRNA. PGC-1β, nuclear respiratory factor-1 (NRF-1) and Mfn1 mRNA as well as cytochrome C oxidase subunit IV (COXIV) mRNA and protein were lower in patients with ALS and ND. Both patient groups had reductions in citrate synthase and cytochrome c oxidase activity. Similar observations were made in skeletal muscle from transgenic ALS G93A transgenic mice. *In vitro*, PGC-1α and PGC-1β regulated *Mfn1* and *Mfn2* in an ERRα-dependent manner. Compared to healthy controls, miRNA 23a, 29b, 206 and 455 were increased in skeletal muscle of ALS patients. miR-23a repressed PGC-1α translation in a 3′ UTR dependent manner. Transgenic mice over expressing miR-23a had a reduction in PGC-1α, cytochome-b and COXIV protein levels. These results show that skeletal muscle mitochondrial dysfunction in ALS patients is associated with a reduction in PGC-1α signalling networks involved in mitochondrial biogenesis and function, as well as increases in several miRNAs potentially implicated in skeletal muscle and neuromuscular junction regeneration. As miR-23a negatively regulates PGC-1α signalling, therapeutic inhibition of miR-23a may be a strategy to rescue PGC-1α activity and ameliorate skeletal muscle mitochondrial function in ALS.

Introduction

Amyotrophic lateral sclerosis (ALS) is a motor neuron disorder resulting in the progressive degeneration of upper and lower motor neurons, a decline in strength, severe muscle atrophy, respiratory insufficiency and death within 3–5 years after the first symptoms (Pasinelli and Brown, 2006). The primary cause of the more frequent sporadic ALS is presently unknown, while in the 5–10% of familial cases (FALS) approximately 25% are associated with mutations in the CuZn superoxide dismutase (CuZn SOD or SOD1) gene. While the general consensus is that ALS is caused by motor neuron death, the precise factor/s causing motor neuron degeneration in ALS remains equivocal (Brooks et al., 2004; Frey et al., 2000; Kennel et al., 1996).

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Perturbations in mitochondrial function are considered an important component in the pathogenesis of ALS (Menzies et al., 2002). In transgenic ALS mice harbouring the G93A-mutated SOD1 gene, the increase in motor neuron mitochondrial degeneration precedes paralysis (Kong and Xu, 1998). This suggests that perturbations in mitochondrial biogenesis, structure and/or function, may play a role in motor neuron degeneration. However, expression of the mutated SOD1 gene in neurons or astrocytes does not lead to ALS (Gong et al., 2000; Pramatarova et al., 2001), suggesting that disease onset and/or progression stems from the reciprocal effects of several cellular systems (Clement et al., 2003). In ALS mice degeneration of the neuromuscular junction (Frey et al., 2000; Kennel et al., 1996) and muscle atrophy and degeneration (Brooks et al., 2004; Marcuzzo et al., 2011) precedes neuronal degeneration. This supports the notion that muscle degeneration may lead and/or contribute to neurodegeneration and play a key role in the cause and/or progression of ALS (Dupuis and Echaniz-Laguna, 2010; Wong and Martin, 2010).

Skeletal muscle of ALS patients and ALS mice presents severe atrophy (Leger et al., 2006) and has considerable mitochondrial disruption and dysfunction, indicated by NADH:CoQ oxidoreductase and cytochrome c oxidase deficiency (Vielhaber et al., 2000; Wiedemann et al., 1998), reduced mitochondrial DNA and reduced levels of mitochondria Mn-SOD (Vielhaber et al., 2000). Disruption of the mitochondrial network enhances skeletal muscle atrophy programs (Romanello et al., 2010), suggesting that impaired mitochondrial function may activate signals that trigger muscle atrophy. It is therefore logical to hypothesise that the pathogenesis and progression of ALS may involve perturbations in signalling molecules that are normally required for the healthy maintenance of skeletal muscle mitochondrial biogenesis and function as well as muscle mass.

The transcriptional co-activators, peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) and PGC-1β positively regulate the mitochondrial network (Finck and Kelly, 2006; Handschin and Spiegelman, 2006) and attenuate muscle atrophy programs (Brault et al., 2010; Sandri et al., 2006). PGC-1α and PGC-1β influence mitochondrial biogenesis and function via the induction and activation of several nuclear transcription factors, such as nuclear respiratory factor-1 (NRF-1) (Wu et al., 1999) and estrogen-related receptor alpha (ERRα) (Mootha et al., 2004; Schreiber et al., 2003, 2004) and several gene targets including mitofusin-2 (Mfn2) (Cartoni et al., 2005; Liesa et al., 2008) and cytochrome C oxidase subunit IV (COX IV) (Lelliott et al., 2006; Puigserver et al., 1998). A down regulation of PGC-1α is observed in conditions associated with mitochondrial dysfunction such as diabetes (Mensink et al., 2007) and ageing (Short et al., 2003) as well as in skeletal muscle atrophy, such as uremia, denervation and cancer cachexia (Sandri et al., 2006). Whether PGC-1α/-1β and members of their transcriptional program involved in mitochondrial biogenesis and function show altered regulation in atrophied skeletal muscle of ALS patients has not been established.

Recently it has been shown that skeletal muscle development and function can be controlled by microRNAs, short (~20–30 nucleotides [nt]) noncoding ribonucleic acids (RNAs) (reviewed previously (Bartel, 2004)). Presently their known functions are to inhibit protein translation or enhance messenger RNA degradation (Hamilton and Baulcombe, 1999; Reinhart et al., 2000). A suite of muscle-enriched miRNAs (miR-1, 133a, 133b, 206; collectively referred to as myomiRs) (Sempere et al., 2004; Small et al., 2010; van Rooij et al., 2007, 2009) have been identified and shown to regulate muscle proliferation and differentiation (reviewed in (Guller and Russell, 2011). Recently, miR-206 has been implicated in the regeneration of neuromuscular synapses in ALS mice (Williams et al., 2009). The regulation of skeletal muscle miRNAs has been investigated in several human muscular disorders (Eisenberg et al., 2007; Gambardella et al., 2010; Greco et al., 2009), but not in skeletal muscle from ALS patients.

The aims of this study were to measure and compare, in skeletal muscle samples from healthy control subjects, patients with ALS and patients with neurogenic disease (ND) (disease control), (1) expression levels of PGC-1α and several of their downstream targets involved in mitochondrial biogenesis and function, including ERRα, NRF-1, Mfn1, Mfn2 and COX IV; (2) mitochondrial enzyme activity and (3) expression levels of miRNAs enriched in skeletal muscle, including miR-1, and 206 and those dysregulated in human muscle disease, including miR-9, -23a, -23b, -29a, -29b, -29c,-31 and -455. A functional miRNA/protein relationship was also investigated *in vitro* using reporter assays and *in vivo* using transgenic mice.

Methods

Subjects

Skeletal muscle biopsies were obtained from fourteen patients diagnosed with ALS (Brooks, 1994), 10 patients diagnosed with neurogenic disease (ND) and 10 age matched healthy control subjects (Table 1). At the time of the biopsy, the duration of illness was 3–48 months. The ND patients consisted of those diagnosed with either SMA type II, post-polio syndrome, motor neuropathy, sensory motor neuropathy or polyneuropathy. The routine histochemical analysis of all biopsies revealed a neurogenic pattern ranging from mild to severe. In all cases, conduction blocks were excluded by sensorimotor nerve conduction studies; laboratory tests ruled out dysproteinemia, hexoaminidase A deficiency, anti-GM1 antibodies, thyroid and parathyroid disorders. The research protocol was approved by the institutional ethics committees and informed consent was obtained from all participants according to the Declaration of Helsinki (BMJ 1991; 302:1194).

Muscle biopsies

For the ALS and ND patients biopsies were taken from the vastus lateralis muscle using either Bergstrom or open biopsy procedures. For the healthy control subjects biopsies were taken from the vastus lateralis muscle using the Bergstrom technique. All biopsies were immediately frozen in liquid nitrogen and stored at −80 °C until analysed.

Animals

ALS transgenic mice

Transgenic mice carrying the SOD1 G93A mutation were compared with control wild-type animals carrying a transgene of the human SOD1 gene (WT) (Jackson Laboratories (Bar Harbor, Maine) (Derave et al., 2003). Thirteen G93A and 10 WT mice were included in the study. Five G93A mice were sacrificed at the age of 90 days (ALS 90) and all other animals were studied at the age of 120 days (ALS 120, n = 8; WT 120, n = 10) (Derave et al., 2003). For the G93A ALS mice 90 and 120 days corresponds to about 65 and 85% respectively, of their life expectancy. All mice were anesthetized by an intraperitoneal infusion of pentobarbitone sodium (50 mg/kg body weight).

miR-23a transgenic mice

The miR-23a transgenic mice have been described (Wada et al., 2011). Genotyping was carried out by PCR and fluorescence microscopy

Table 1
Characteristics of subjects included in the study.

	Control	ALS	ND
Patients (n)	10	14	10
M/F	7/3	10/4	7/3
Age (years)			
Mean ± SD	53 ± 17	59 ± 9	53 ± 13
Range	25–73	33–79	20–71

M = male; F = female.