Behavioural Brain Research 313 (2016) 272–281

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Lack of age-related clinical progression in PGC-1α-deficient mice – implications for mitochondrial encephalopathies

Levente Szalardy[a], Mate Molnar[a], Rita Torok[a], Denes Zadori[a], Gabor G. Kovacs[b], Laszlo Vecsei[a,c], Peter Klivenyi[a,*]

[a] Department of Neurology, Faculty of Medicine, Albert Szent-Györgyi Clinical Center, University of Szeged, H-6725 Szeged, Semmelweis u. 6, Hungary
[b] Institute of Neurology, Medical University of Vienna, AKH 4J, A-1090 Vienna, Währinger Gürtel 18-20, Austria
[c] MTA-SZTE Neuroscience Research Group, H-6725 Szeged, Semmelweis u. 6, Hungary

HIGHLIGHTS

- FL-PGC-1α -/- mice have a symptomatology corresponding with mitochondrial encephalopathy.
- The behavioral alterations intriguingly do not progress with age.
- The alterations do not associate with retinal or spinal cord involvement.
- The histopathology of skeletal muscle demonstrates only mild myopathic changes.
- CNS-specific isoforms are the predominant PGC-1α mRNAs in the murine brain.

ARTICLE INFO

Article history:
Received 17 April 2016
Received in revised form 9 July 2016
Accepted 13 July 2016
Available online 15 July 2016

Keywords:
Behavior
Isoforms
Pathology
PGC-1α
Phenotype
Progression

ABSTRACT

Impaired peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α) function has been demonstrated in several neurodegenerative diseases, and murine whole-body knockouts of PGC-1α have been considered as models for Huntington's disease. Recent neuropathological studies, however, rather propose these animals to be morphological models of mitochondrial encephalopathies, with special reminiscence of Kearns-Sayre syndrome. PGC-1α-deficient animals have already been subjected to behavioral assessments; however, the contradictory findings and the paucity of data assessing long-term progression necessitated further examinations. This study provides a comprehensive neurological phenotypic profiling of full-length-(FL-)PGC-1α-deficient mice in a broad age spectrum, with special focus on previously controversial findings, the issue of long-term phenotypic progression, the histopathological assessment of previously non-characterized tissues of potential clinicopathological relevance, and the gene expression profile of novel brain-specific isoforms of PGC-1α. Our findings demonstrate moderate hypomotility with signs of gait and trunk ataxia in addition to severe impairments in coordination and muscle strength in FL-PGC-1α-deficient mice, phenotypic features consistent of a mitochondrial disease. Intriguingly, however, these early alterations did not progress with age, the understanding of which may

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CNS, central nervous system; COX, cythochrome oxidase; ERR, estrogen-related receptor; FL-PGC-1α, full-length PGC-1α; GFAP, glial fibrillary acidic protein; HE, hematoxylin and eosin; HD, Huntington's disease; KLB, Klüver-Barrera staining; KSS, Kearns-Sayre syndrome; LS, Leigh syndrome; MEF2C, myocyte-specific enhancer factor 2C; MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; MERRF, myoclonic epilepsy with ragged-red fibers; MNGIE, mitochondrial neurogastrointestinal encephalopathy; NARP, neuropathy, ataxia, retinitis pigmentosa; NRF-1, and -2, nuclear respiratory factor 1 and 2; NT-PGC-1α, N-terminal fragment of PGC-1α; PPARγ, peroxisome proliferator-activated receptor-gamma; PD, Parkinson's disease; PGC-1α, PPARγ coactivator 1-alpha; RT-PCR, real-time polymerase chain reaction.

* Corresponding author.
E-mail addresses: levente.szalardy@yahoo.com (L. Szalardy), matem87@gmail.com (M. Molnar), torok.rituska@gmail.com (R. Torok), zadorid@gmail.com (D. Zadori), gabor.kovacs@meduniwien.ac.at (G.G. Kovacs), vecsei.laszlo@med.u-szeged.hu (L. Vecsei), klivenyi.peter@med.u-szeged.hu (P. Klivenyi).

http://dx.doi.org/10.1016/j.bbr.2016.07.021

unveil mechanisms of potential therapeutic relevance, as discussed. The observed phenotype did not associate with retinal or spinal cord alterations, and was accompanied by mild myopathic changes. Based on these, FL-PGC-1α-deficient mice can be regarded not only as morphological but behavioral models of mitochondrial encephalopathies, with an important temporal limitation that has now been clarified. The mechanisms capable of halting a potentially lethal phenotype are to be unveiled, as they may hold therapeutic value for mitochondrial diseases.

1. Introduction

Impaired mitochondrial function has widely been linked to the development of various neurodegenerative disorders, including Parkinson's disease (PD), Huntington's disease (HD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS) [1–4]. These disorders are characterized by a relatively selective loss of neurons in different central nervous system (CNS) areas, corresponding to the leading clinical manifestations, in most of them with specific motor impairments. In addition, alterations of either nuclear or mitochondrial genome affecting mitochondrial functions at various levels are pathognomonic of a group of congenital multi-systemic diseases, collectively termed mitochondrial encephalopathies [5–8]. These diseases that include Kearns-Sayre syndrome (KSS), Leigh syndrome (LS), mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged-red fibers (MERRF), neuropathy, ataxia, retinitis pigmentosa (NARP), and mitochondrial neurogastrointestinal encephalopathy (MNGIE) generally present with a progressive symptomatology corresponding to the multisystemic involvement of the brain, skeletal muscles, heart, and liver, among other less commonly affected organs [5–8]. The involvement of the brain manifests in different forms of mitochondrial encephalopathy in the different syndromes, commonly characterized by various extents of white matter vacuolation accompanied by reactive astrogliosis with or without apparent selective neurodegeneration in particular brain regions.

Several lines of evidence indicate that peroxisome proliferator-activated receptor-gamma (PPARγ) coactivator 1-alpha (PGC-1α), a nuclear-encoded coactivator of an armada of transcriptional factors that play extensive roles in the activation of adaptive mitochondrial responses, may contribute to the mitochondrial dysfunction in neurodegenerative disorders, including PD, AD, HD, and ALS [1–4]. The coactivation of genes such as nuclear respiratory factor 1 and 2 (NRF-1, -2), estrogen-related receptors (ERRs), myocyte-specific enhancer factor 2C (MEF2C), and PPARs results in an enhanced expression of a wide spectrum of proteins involved in mitochondrial function and biogenesis [9], including mitochondrial replication and transcription, the import and assembly of respiratory complex subunits, a tissue-dependent induction of oxidative phosphorylation and thermoregulation, the enhancement of gluconeogenesis and fatty acid oxidation, as well as the increase of defense against oxidative stress [10,11]. In line with these, experimental evidence suggests that pharmacological or transcriptional activation of PGC-1α may hold therapeutic value in neurodegenerative as well as mitochondrial diseases [11–13].

Despite its broad roles in mitochondrial functions, the absence of PGC-1α is compatible with life. The first pioneering publications with two independent murine PGC-1α knockout strains commonly described signs of striatal degeneration, proposing that these animals might model HD [14,15], which corresponded with the findings of reduced striatal expression of PGC-1α and its target genes in the striatum of HD patients [16,17] as well as in its transgenic *in vivo* [16–18] and *in vitro* models [16,17] (comprehensively reviewed in [11]). Not doubting the potentially essential role of PGC-1α dysfunction in the pathogenesis of HD, the concept that PGC-1α-deficient animals themselves might model HD, however, has recently been questioned by serial findings of independent morphological and molecular biological analyses [19–21]. One of the two pioneering publications reported the development of a complete (*i.e.*, with no residual expression) PGC-1α whole-body knockout strain, exhibiting hyperactivity with no overt muscle phenotype [14]. Though PGC-1α has recently been demonstrated to have a number of previously unknown isoforms [22], to the current knowledge, this strain can still be regarded as a complete knockout of PGC-1α [23]. Contrastingly, the second pioneering publication reported the development of another whole-body knockout strain demonstrating hypomotility and weakness [15], a strain later turned out to express an N-terminal fragment of PGC-1α (NT-254, functionally identical with the 267 amino acid-long N-truncated splice variant, NT-PGC-1α) but not the full-length protein (FL-PGC-1α) [24]. This was then followed by reports on muscle-specific complete PGC-1α knockouts with myopathic signs and weakness [25], as well as on complete whole-body and brain-specific PGC-1α knockouts failing to recapitulate hyperactive behavior [20,26] but demonstrating impaired coordination [20] and ataxia proposed to be of cerebellar type [27]. Notably, the increasing behavioral data from the complete knockout phenotypes tend to delineate a phenotype resembling a relatively compensated mitochondrial disease. Correspondingly, recent neuropathological analyses on FL-PGC-1α -/- mice reported wide-spread, locally dramatic vacuolation of the white matter, predominantly affecting the thalamus, basal ganglia, internal capsule, pontomedullary brainstem as well as the cerebellum, accompanied by reactive astrogliosis in the brainstem and cerebellar nuclei [19]. In fact, this pathology is highly reminiscent of that seen in a human mitochondrial spongiform encephalopathy, with particular resemblance to KSS [21]. Notably, no morphological correlates of striatal axonal or neuronal degeneration were apparent in FL-PGC-1α -/- animals [19] even at 75 weeks of age [21], which corresponds with earlier findings on young (postnatal day 10) FL-PGC-1α -/- animals *via* immunohistochemistry of striatal neurofilament [28] and has recently been confirmed by Lucas et al. demonstrating no significant loss of medium-sized spiny neurons *via* molecular biological methods in complete PGC-1α -/- mice [20]. Of note, the same group reported their observation of a relatively stable phenotype in complete PGC-1α -/- mice, with no robust progression between 4 and 12 weeks of age [20].

All these above detailed contradictory findings on PGC-1α-deficient mice and the recent report of a relatively stable phenotype in young PGC-1α -/- animals prompted us to perform a comprehensive profiling of motor phenotype of FL-PGC-1α -/- mice bred in our institute through a wide spectrum of age, with a special focus on the issue of phenotypic progression. The manuscript also addresses the expression profile of novel CNS-specific isoforms of PGC-1α in FL-PGC-1α -/- mice (containing exon B4) [29], a feature previously not characterized in this strain. As a supplement of prior published neuropathological findings on adult [19] and aged [21] FL-PGC-1α -/- brains, additional neuropathological work-up has been performed to better understand the potential background