BBA - Molecular Basis of Disease 1864 (2018) 3588–3597

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

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

Mitochondrial dysfunction in fibroblasts of Multiple System Atrophy

Giacomo Monzio Compagnoni[a], Giulio Kleiner[b], Andreina Bordoni[a], Francesco Fortunato[a], Dario Ronchi[a], Sabrina Salani[a], Marianna Guida[c], Corrado Corti[c], Irene Pichler[c], Christian Bergamini[d], Romana Fato[d], Maria Teresa Pellecchia[e], Annamaria Vallelunga[e], Francesca Del Sorbo[f], Antonio Elia[f], Chiara Reale[g], Barbara Garavaglia[g], Gabriele Mora[h], Alberto Albanese[i], Filippo Cogiamanian[j], Gianluca Ardolino[j], Nereo Bresolin[a], Stefania Corti[a], Giacomo P. Comi[a], Catarina M. Quinzii[b], Alessio Di Fonzo[a,*]

[a] IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy
[b] Department of Neurology, Columbia University, New York 10032, NY, USA
[c] Institute for Biomedicine, Eurac Research, Via Galvani 31, 39100 Bolzano, Italy
[d] Department of Pharmacy and Biotechnology (FaBiT), University of Bologna, Bologna, Italy
[e] Neuroscience Section, Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Italy
[f] Neurology Unit I, Neurological Institute "C. Besta" IRCCS Foundation, Milan, Italy
[g] Medical Genetics and Neurogenetics Unit, IRCCS Foundation Istituto Neurologico Carlo Besta, Milan, Italy
[h] Department of Neurological Rehabilitation, ICS Maugeri, IRCCS, Istituto Scientifico di Milano, Milan, Italy
[i] Department of Neurology, Humanitas Research Hospital, Rozzano, Milan, Italy
[j] U.O. Neurofisiopatologia, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

ARTICLE INFO

Keywords:
Multiple System Atrophy
Cellular models
Fibroblasts
Mitochondria

ABSTRACT

Multiple System Atrophy is a severe neurodegenerative disorder which is characterized by a variable clinical presentation and a broad neuropathological spectrum. The pathogenic mechanisms are almost completely unknown. In the present study, we established a cellular model of MSA by using fibroblasts' primary cultures and performed several experiments to investigate the causative mechanisms of the disease, with a particular focus on mitochondrial functioning.

Fibroblasts' analyses (7 MSA-P, 7 MSA-C and 6 healthy controls) displayed several anomalies in patients: an impairment of respiratory chain activity, in particular for succinate Coenzyme Q reductase ($p < 0.05$), and a reduction of complex II steady-state level ($p < 0.01$); a reduction of Coenzyme Q10 level ($p < 0.001$) and an up-regulation of some CoQ10 biosynthesis enzymes, namely COQ5 and COQ7; an impairment of mitophagy, demonstrated by a decreased reduction of mitochondrial markers after mitochondrial inner membrane depolarization ($p < 0.05$); a reduced basal autophagic activity, shown by a decreased level of LC3 II ($p < 0.05$); an increased mitochondrial mass in MSA-C, demonstrated by higher TOMM20 levels ($p < 0.05$) and suggested by a wide analysis of mitochondrial DNA content in blood of large cohorts of patients.

The present study contributes to understand the causative mechanisms of Multiple System Atrophy. In particular, the observed impairment of respiratory chain activity, mitophagy and Coenzyme Q10 biosynthesis suggests that mitochondrial dysfunction plays a crucial role in the pathogenesis of the disease. Furthermore, these findings will hopefully contribute to identify novel therapeutic targets for this still incurable disorder.

* Corresponding author at: IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Via Francesco Sforza 35, 20122 Milan, Italy.

E-mail addresses: giacomo.monzio@unimi.it (G. Monzio Compagnoni), gk2438@cumc.columbia.edu (G. Kleiner), andreina.bordoni@unimi.it (A. Bordoni), francesco.fortunato@unimi.it (F. Fortunato), M.Guida@eco-research.it (M. Guida), Corrado.Corti@eurac.edu (C. Corti), Irene.Pichler@eurac.edu (I. Pichler), christian.bergamini2@unibo.it (C. Bergamini), romana.fato@unibo.it (R. Fato), mpellecchia@unisa.it (M.T. Pellecchia), avallelunga@unisa.it (A. Vallelunga), Francesca.DelSorbo@istituto-besta.it (F. Del Sorbo), antonio.elia@istituto-besta.it (A. Elia), chiara.reale@istituto-besta.it (C. Reale), Barbara.Garavaglia@istituto-besta.it (B. Garavaglia), gabriele.mora@fsm.it (G. Mora), alberto.albanese@humanitas.it (A. Albanese), nereo.bresolin@unimi.it (N. Bresolin), stefania.corti@unimi.it (S. Corti), giacomo.comi@unimi.it (G.P. Comi), cmq2101@cumc.columbia.edu (C.M. Quinzii), alessio.difonzo@policlinico.mi.it (A. Di Fonzo).

https://doi.org/10.1016/j.bbadis.2018.09.018
Received 6 June 2018; Received in revised form 7 September 2018; Accepted 17 September 2018
Available online 19 September 2018
0925-4439/ © 2018 Published by Elsevier B.V.

1. Introduction

Multiple System Atrophy (MSA) is a severe neurodegenerative disorder which affects several areas of the Nervous System. MSA is clinically characterized by variable degrees of parkinsonism, cerebellar ataxia, dysautonomia and pyramidal features. A parkinsonian (MSA-P) and a cerebellar (MSA-C) subtype can be distinguished according to the predominant symptomatology [1,2].

No effective therapies are available, although many pharmacological strategies are under investigation [3].

The heterogeneity of MSA clinical presentation correlates with the numerous regions of the nervous system which are affected, including striato-nigral pathway, cerebellum and ponto-cerebellar fibers, brainstem and autonomous nervous system. Neuropathologically, the disease is mainly characterized by the finding of protein aggregates (glial cytoplasmic inclusions, GCIs) in oligodendrocytes. As alpha-synuclein is the main component of these inclusions, MSA is classified, together with Parkinson's disease and Dementia with Lewy Bodies, as an alpha-synucleinopathy [4,5].

The pathogenic mechanisms of the disease are almost completely unclear, although several hypotheses have been proposed. In particular, most of the studies have focused on alpha-synuclein over-expression or cell-to-cell transfer, inflammation and mitochondrial functioning [5].

Mitochondrial dysfunction has been demonstrated to be implicated in synucleinopathies. Although most of the studies have focused on Parkinson's disease [6], Multiple System Atrophy has been investigated, too. Previous studies assessed the activity level of respiratory chain in MSA patients and healthy controls, detecting a reduced complex I activity in patients' skeletal muscle [7] but not in platelets or substantia nigra [8]. No significant differences were observed in the amount of mtDNA deletions or rearrangements in substantia nigra of MSA patients compared to controls [9]. More interest was raised by a study which suggested a causative role of mutations in *COQ2*, encoding the second enzyme involved in Coenzyme Q10 (CoQ10) biosynthesis [10]. However, this result was not confirmed by other investigators [11–13] and the issue remains controversial. Of note, recessive *COQ2* mutations cause primary CoQ10 deficiency leading to an infantile encephalomyopathy/nephropathy with cerebellar atrophy and other primary CoQ10 deficiency syndromes are characterized by cerebellar ataxia as main clinical feature [14,15]. Finally, a reduction of CoQ10 amount selectively in cerebellum of MSA patients, but not in other brain areas, was reported in two independent studies [16,17] and a reduced CoQ10 level was described in patients' plasma, too [18–20].

The purpose of the present work is to investigate the pathogenic mechanisms of MSA by using fibroblasts derived from patients and controls, with a particular focus on mitochondrial functioning. In particular, we focused our attention on respiratory chain function and oxygen consumption, mitochondrial mass (in both fibroblasts and peripheral blood cells), mitophagy, autophagy, CoQ10 amount and biosynthesis.

2. Materials and methods

2.1. Ethical issues

All the experiments were performed in compliance with the Helsinki declaration and informed consent was obtained from all the subjects involved in the study.

2.2. Patients selection and skin biopsies withdrawal

Skin biopsies were obtained from 7 MSA-P patients, 7 MSA-C patients and 6 age-matched healthy controls. Seventeen samples were obtained from the Italian movement disorders outpatient service of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan), where biopsies were withdrawn from the ventral surface of the right arm through a 5 mm punch. Three fibroblast cell lines were obtained from the Italian Telethon Network of Genetic Biobanks [21]. Details about age and sex of each subject are reported in Table S1.

Diagnosis of probable MSA was performed in compliance with widely accepted clinical criteria (Gilman et al., 2008) [22].

2.3. PBCs samples

DNA extracted from peripheral blood cells of patients affected with MSA was obtained from three Italian movement disorders outpatient services. Details about the composition of each group are reported in Table S2.

2.4. Fibroblasts culture

Fibroblasts were isolated from skin biopsies and expanded in Dulbecco's modified eagle medium supplemented with fetal bovine serum (15%), penicillin/streptomycin (1%) and amphotericin B (1%).

2.5. Spectrophotometric analyses

Protein extraction was performed by sonication (50 W for 10 s, 3 times) after resuspending cell pellets in proper buffer (pH 7.2). Lysates were centrifuged at 750*g* for 10 min and supernatant was recovered. Lowry method was used for protein quantification. A Lambda 2 Parkin Elmer spectrophotometer was used to assess enzymatic activities. Analyses were performed at specific wave lengths for each enzymatic activity after preparing proper solutions as previously described [23] with mild changes. NADH dyhydrogenase (340 nm): H_2O (455 μl), K-phosphate pH 7.5 0.1 M (340 μl), $K_3[Fe(CN)_6]$ 17 mM (100 μl), NADH 2 mM (100 μl), homogenate (5 μl). NADH ubiquinone 1 reductase (340 nm): H_2O (610 μl), K-phosphate pH 7.5 0.1 M (200 μl), albumin 1% (100 μl), NADH 2 mM (70 μl), homogenate (10 μl), sodium azide 100 mM (10 μl), CoQ1 6 mM (5 μl); activity measured after rotenone administration (1 mM, 5 μl) was subtracted. Succinate dehydrogenase (SDH) (600 nm): K-phosphate pH 7 0.1 M (500 μl), 2,6 dichloroindophenol 0.5 mM (200 μl), H_2O (130 μl), succinic acid 200 mM (100 μl), KCN 30 mM (50 μl), homogenate (20 μl). Succinate CoQ Reductase (600 nm): K-phosphate pH 7 0.1 M (500 μl), 2,6 dichloroindophenol 0.5 mM (200 μl), H_2O (127 μl), succinic acid 200 mM (100 μl), KCN 30 mM (50 μl), homogenate (20 μl), CoQ1 15 mM (3 μl). NADH cytochrome *C* reductase (550 nm): H_2O (490 μl), K-phosphate pH 7.5 0.1 M (250 μl), NADH 2 mM (120 μl), cytochrome *C* 1 mM (100 μl), KCN 30 mM (20 μl), homogenate (20 μl). Succinate cytochrome *C* reductase (550 nm): K-phosphate pH 7.5 0.1 M (500 μl), H_2O (300 μl), cytochrome *C* 1 mM (100 μl), succinate 400 mM (50 μl), homogenate (30 μl), KCN 30 mM (20 μl). Cytochrome oxidase (550 nm): H_2O (680 μl), K-phosphate pH 7 0.1 M (200 μl), reduced cytochrome *C* 1% (100 μl), homogenate (20 μl). Citrate synthase (412 nm): H_2O (800 μl), DTNB 1 mM (100 μl), oxaloacetic acid 10 mM (50 μl), AcetylCoA 10 mM (30 μl), homogenate (20 μl). Experiments were performed at 30 °C. Analyses were performed through a Parkin Elmer software. Measurements were normalized over the activity level of citrate synthase, a stable matrix mitochondrial enzyme; this latter step was performed in order to normalize respiratory chain activity over mitochondrial mass.

2.6. High resolution respirometry

A high-resolution respirometry Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria) was used to assess oxygen consumption in fibroblasts [24]. For each analysis, 800,000 cells were resuspended in proper medium (MiRO5) and injected into the instrument's chambers. Oxygen flux was measured at basal level and after the progressive administration of several compounds: digitonin (4.1 μM), malate (2 mM) and glutamate (10 mM), ADP (1–5 mM),

Download English Version:

https://daneshyari.com/en/article/10156636

Download Persian Version:

https://daneshyari.com/article/10156636

Daneshyari.com