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Oct-1 recruitment to the nuclear envelope in adult-onset autosomal dominant leukodystrophy

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ABSTRACT

Adult-onset autosomal dominant leukodystrophy (ADLD) is a slowly progressive neurological disorder characterised by pyramidal, cerebellar, and autonomic disturbances. Duplication of the *LMNB1* gene is the genetic cause of ADLD, yet the pathogenetic mechanism is not defined. In this study, we analysed cells and muscle tissue from three patients affected by ADLD, carrying an extra copy of the *LMNB1* gene. Lamin B1 levels were dramatically increased in ADLD nuclei, both in skin fibroblasts and skeletal muscle fibres. Since lamin B1 is known to bind Oct-1, a transcription factor involved in the oxidative stress pathway, we investigated Oct-1 fate in ADLD. Oct-1 recruitment to the nuclear periphery was increased in ADLD cells, while nucleoplasmic localisation of the transcription factor under oxidative stress conditions was reduced. Importantly, lamin B1 degradation occurring in some, but not all ADLD cell lines, slowed down lamin B1 and Oct-1 accumulation. In skeletal muscle, focal disorganisation of sarcomeres was observed, while IIB-myosin heavy chain, an Oct-1 target gene, was under-expressed and rod-containing fibres were formed. These data show that a high degree of regulation of lamin B1 expression is implicated in the different clinical phenotypes observed in ADLD and show that altered Oct-1 nuclear localisation contributes to the disease phenotype.

1. Introduction

The hereditary leukodystrophies represent a rare group of neurological disorders, in which partial or total dysmyelination occurs in either the central nervous system (CNS), the peripheral nervous system or both [1]. Leukodystrophies include adult-onset autosomal dominant leukodystrophy (ADLD, OMIM #169500) first described in 1984 in a large Irish American family [2]. ADLD, a very rare disease, is characterised by a slowly progressive central nervous system myelin degeneration with pyramidal, cerebellar and autonomic disturbances [3,4]. The symptoms begin between the forth to sixth decades of life with early presentation of autonomic symptoms, orthostatic hypotension and decreased sweating [4,5]. Autonomic symptoms precede cerebellar signs and pyramidal abnormalities [6]. ADLD is caused by duplication of *LMNB1* gene encoding lamin B1 [7] and represents the first example of the involvement of lamins in disorders of the CNS with severe demyelinating phenotype [7,8]. Lamin B1 is a component of nuclear lamina which is a fibrous meshwork of intermediate filaments underlying the inner nuclear membrane [9]. The nuclear lamina is made up of two major types of lamins, A and B type, based on their sequence homologies, expression patterns, structural features and biochemical and dynamic properties. The A-type lamins, including lamins A and C, are derived from a single gene (*LMNA*) by alternative splicing and are expressed in differentiated cells [10]. The B-type lamins, lamin B1 and B2 are encoded by two separate genes, *LMNB1* and *LMNB2* and are present in all cell types including stem cells [10,11]. Lamin B3 is also encoded by the *LMNB2* gene and it is expressed in germ cells only [12]. Lamin A and B type lamins are translated as precursors and require post-translational modifications of their carboxyterminal CAAX box to become mature lamins [13]. In contrast to lamin A, which lacks the carboxy-terminal farnesyl and carboxymethyl modifications in the mature isoform, B-type lamins remain permanently farnesylated

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and carboxymethylated [14]. The nuclear lamina provides structural support and plays dynamic roles in the organisation and regulation of chromatin, transcription, DNA repair, DNA replication and epigenetic phenomena of chromatin transitions [10]. The importance of the nuclear lamina in normal cellular functioning has been supported by the identification of a wide range of human diseases, collectively called laminopathies, caused by mutations in *LMNA* or other genes encoding proteins of the nuclear envelope [10]. More than 300 human mutations have been identified in the *LMNA* gene and a wide range of clinical phenotypes have been associated with lamin A/C defects including disorders of the musculoskeletal system, premature aging, fat metabolism and peripheral nervous system. Two diseases have been linked to mutations in *LMNB1* or *LMNB2*. Besides ADLD, a study reported an increased frequency of *LMNB2* polymorphisms in patients with acquired partial lypodystrophy [15].

An important mechanism by which the lamins can regulate gene expression is by associating with specific transcription factors such as AP1, c-Fos, MOK2, sterol response element binding protein 1 (SREBP1) [10,16] and the octamer transcription factor (Oct-1) [17]. Lamins are also associated with transcription factors indirectly via several lamin-binding proteins including emerin and LAP2 [10]. OCT-1 is a ubiquitous transcription factor with activating and silencing activities and is widely expressed; moreover it is a potent regulator of metabolism and tumourigenicity [18]. In fact, Oct-1 interacts with BRCA1, a tumour suppressor protein associated with DNA damage response [19]. The association of Oct-1 with lamin B1 at the nuclear envelope appears to be important for the oxidative stress response in MEFs, as lamin B1 deficiency leads to a dysregulation of Oct-1-dependent genes and to an increase in reactive oxygen species [17]. In the study reported in the present paper, performed on human skeletal muscle tissue and human fibroblast cultures from ADLD patients, we show that ADLD nuclei from diverse affected subjects express different levels of lamin B1, irrespective of the gene duplication event. We find that differences in lamin B1 levels are elicited by different activation of a degradation mechanism, which is differently efficient in patients. Moreover, recruitment of Oct-1 to the nuclear periphery parallels lamin B1 accumulation in the nuclear lamina. Downstream of this event, IIB-myosin heavy chain (IIB-MyHC), a target of Oct-1 activation, is downregulated to undetectable levels in ADLD1 muscle. These data contribute to the understanding of the different clinical phenotypes observed in ADLD.

2. Materials and methods

2.1. Clinical phenotype

2.1.1. Patient 1 (ADLD1)

A 53-year-old man carrying the *LMNB1* duplication was evaluated after two years history of autonomic disturbances (urinary urgency, ejaculatory failure, and orthostatic intolerance) followed by progressive lower limb fatigue and poor balance. On admission revealed a symptomatic orthostatic hypotension, cerebellar and pyramidal signs. Cardiovascular reflexes indicated a selective sympathetic failure, sparing cardiovagal function and microneurographic recordings showed absent muscle sympathetic nerve activity. The evaluation of autonomic innervation of skin annexes revealed abnormal noradrenergic dopamine-β-hydroxylase (DβH) immunoreactive fibres and preserved cholinergic vasoactive intestinal polypeptide (VIP) immunoreactive fibres.

2.1.2. Patient 2 (ADLD2)

A 46-year-old man carrying the *LMNB1* duplication complains initial erectile dysfunction. Cardiovascular reflexes indicated a selective sympathetic failure (asymptomatic orthostatic hypotension), sparing cardiovagal function and microneurographic recordings showed absent muscle sympathetic nerve activity. The evaluation of autonomic innervation of skin annexes revealed abnormal noradrenergic dopamine-β-hydroxylase (DβH) immunoreactive fibres and preserved cholinergic vasoactive intestinal polypeptide (VIP) immunoreactive fibres.

2.1.3. Patient 3 (ADLD3)

A 57-year-old woman carrying the *LMNB1* duplication was examined after seven years history of slowly progressive clumsy gait and urinary urgency. Neurological examination showed pyramidal signs. Cardiovascular reflexes showed borderline results. Skin biopsy revealed normal findings.

In all patients, brain MRI showed a diffuse T2 hyperintensity of the cerebral white matter with a predominant frontoparietal distribution and the cardiac autonomic nervous system assessed by means of 123-iodine metaiodobenzylguanidine (123I MIBG) was unremarkable.

The patients underwent an extensive work-up to exclude a demyelinating disorder caused by a known metabolic defect. Lysosomal enzyme activities were normal (exosoaminidase A and B, arysulfatase A, galactosyl-cerebrosidase, alpha and beta mannosidase) as well as the very long-chain fatty acids.

2.2. Gene duplication and array-based comparative genomic hybridisation

Genomic DNA was isolated from three confluent fibroblast cultures derived from ADLD patients, using the DNeasy Blood & Tissue Kit (QIAGEN GmbH, Hilden, Germany) according to standard methods. The presence of *LMNB1* duplication was assayed through multiplex PCR coupled with semi-quantitative denaturing high performance liquid chromatography (DHPLC)-based technique.

Moreover, array-Based Comparative Genomic Hybridisation (array-CGH) was performed using a whole genome high resolution platform (CytoChip Oligo ISCA 180 K, BlueGnome, Cambridge, UK). This array consists of 60-mer oligonucleotides spaced at ~25 kb over the full genome. The positions of oligomers refer to the Human Genome March 2006 assembly (hg18). A sex-matched reference DNA (Promega, Madison, UK) has been used for each sample tested. Digestion, labeling and hybridisation were performed following the manufacturer's protocol (version 1.2) (www.cytochip.com). Slides were scanned using an Agilent scanner, with a 5 μm resolution. Data were analysed using BlueFuse Multi Software (BlueGnome, Cambridge, UK).

2.3. Gene expression analysis

Total RNA was isolated using Rneasy Mini Kit (Qiagen GmbH, Hilden, Germany) from confluent fibroblast cultures following manufacturer instructions. RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Monza, Italy). *LMNB1* expression was evaluated by Real Time PCR, performed using an Applied Biosystems StepOne thermal cycler instrumentation (Applied Biosystems), by amplifying 1 μg of cDNA and the TaqMan Gene Expression Assays (Applied Biosystems). Probes and primers obtained by Applied Biosystems were: GAPDH, assay ID Hs99999905_m1, *LMNB1*, assay ID Hs01059210_m1. The amplification protocol was: 50 °C for 2 min; 95 °C for 10 min; 95 °C for 15 s, 60 °C for 1 min, for 40 cycles. The results were calculated by the 2-ΔΔCT method. The experiments were performed in quadruplicate.

2.4. Cell culture and treatment

Skin and muscle biopsies were obtained from three consenting ADLD patients of a large Italian family [6] and were used to establish primary skin fibroblasts cultures or cryosectioned to analyze protein localization in mature muscle [20]. Tibialis anterior muscle was subjected to biopsy. Control muscle biopsies were also obtained from patients undergoing surgery. All the local and EU ethical issues were respected.

To block lysosomal activity, chloroquine (Sigma, 25 μM for 20 hours or 100 μM for 8 hours) was applied. To check proteasome-mediated