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Neuromuscular Disorders 23 (2013) 75–83

www.elsevier.com/locate/nmd

Expression of myogenic regulatory factors and myo-endothelial remodeling in sporadic inclusion body myositis

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Received 22 February 2012; received in revised form 4 September 2012; accepted 13 September 2012

Abstract

Muscle repair relies on coordinated activation and differentiation of satellite cells, a process that is unable to counterbalance progressive degeneration in sporadic inclusion body myositis (s-IBM). To explore features of myo regeneration, the expression of myogenic regulatory factors Pax7, MyoD and Myogenin and markers of regenerating fibers was analyzed by immunohistochemistry in s-IBM muscle compared with polymyositis, dermatomyositis, muscular dystrophy and age-matched controls. In addition, the capillary density and number of interstitial $CD34^+$ hematopoietic progenitor cells was determined by double-immunoflourescence staining. Satellite cells and regenerating fibers were significantly increased in s-IBM similar to other inflammatory myopathies and correlated with the intensity of inflammation ($R > 0.428$). Expression of MyoD, visualizing activated satellite cells and proliferating myoblasts, was lower in s-IBM compared to polymyosits. In contrast, Myogenin a marker of myogenic cell differentiation was strongly up-regulated in s-IBM muscle. The microvascular architecture in s-IBM was distorted, although the capillary density was normal. Notably, $CD34^+$ hematopoietic cells were significantly increased in the interstitial compartment. Our findings indicate profound myo-endothelial remodeling of s-IBM muscle concomitant to inflammation. An altered expression of myogenic regulatory factors involved in satellite cell activation and differentiation, however, might reflect perturbations of muscle repair in s-IBM.

Keywords: Sporadic inclusion body myositis; Myogenesis; Satellite cells; Microvascularization

1. Introduction

Sporadic inclusion body myositis (s-IBM) is an acquired myopathy that occurs mostly in patients above the age of 50 years [1]. Recently, the prevalence rate of s-IBM, age and sex-adjusted to the US census count in 2000, was estimated at 7.06 cases per 100,000 [2]. The disease manifests with progressive asymmetric weakness and atrophy of proximal and distal limb muscles [3] that are resistant to immunosuppressive treatment [4]. Current concepts on the underlying pathogenic mechanisms of s-IBM have focused on the unique combination of endomysial inflammation [5,6] and myo-degeneration related to impaired

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http://dx.doi.org/10.1016/j.nmd.2012.09.003

degradation and abnormal aggregation of amyloid and related proteins [7] that delineate s-IBM from other inflammatory myopathies (IM) [8]. The understanding of triggering events and pathogenic pathways that confer muscle fiber injury in s-IBM, however, remains incomplete [9].

Maintenance and repair of skeletal muscle relies mainly on the proliferative potential of satellite cells (SCs), a distinct population of committed myogenic progenitors also called adult muscle stem cells, that reside in sublaminal cell niches attached to mature myofibers [10]. In adult muscle, SCs represent approximately 5% of the total myonuclei [11,12] and remain in a quiescent state expressing the paired box transcription factor Pax7, necessary for their maintenance during postnatal life [13]. Muscle damage activates SCs to proliferate, which subsequently differentiate and form fusion-competent myoblasts in order to repair or replace damaged muscle fibers [11,14]. This highly coordinated myogenic pathway is controlled by myogenic regulatory factors Myf5, Mrf4, MyoD, and Myogenin, a group of nuclear transcription factors, which are sequentially expressed during muscle regeneration [15]. Following myoblast fusion, emerging muscle fibers transiently up-regulate developmental proteins such as embryonic and neonatal myosin heavy chains [15], the intermediate filament protein vimentin [16] and neural cell adhesion molecule (NCAM) that is widely expressed in SCs and regenerating as well as denervated muscle fibers [17]. Finally, maturation and functional re-integration of newly regenerated fibers critically depend on the reorganization of supporting structures in the extracellular compartment [15] and sufficient revascularization at the site of injury [18].

Muscle repair in s-IBM is unable to prevent progressive loss of muscle fibers, although induction of myogenesis has been indicated by the observation of an increased number of $Pax7^{+}$ satellite cells [19] and regenerating fibers re-expressing developmental molecules in muscle biopsies from a small number of s-IBM patients [16,20]. *In vitro* studies, in contrast, demonstrated a reduced proliferation rate of s-IBM myoblasts versus age-matched controls and the authors proposed that a defective regeneration of s-IBM muscle might be a contributory factor to the complex pathophysiology of the disease [21]. The purpose of the present study was to explore the *in situ* expression of myogenic regulatory factors, the frequency and distribution of regenerating fibers and the pattern of microvascularisation in muscle biopsies from a large series of patients in order to analyze potential perturbations of the myogenic program and consecutive tissue remodeling in s-IBM skeletal muscle in an *in vivo* context. Results were compared with polymyositis and dermatomyositis, muscular dystrophies and normal aged muscle and were related to the age of patients, duration of the disease and to the extent of inflammation.

2. Materials and methods

2.1. Patients and biopsy specimens

Medical and pathological records from all patients with clinically suspected s-IBM who were seen at the Reference Center for Neuromuscular Diseases, Institut de Myologie, Hôpital Pitié-Salpetrière [4] and the Division for Neuromuscular Diseases at the Department of Neurology, Innsbruck Medical University between 1999 and 2008 were retrospectively reviewed. All patients had a muscle biopsy for diagnostic purposes after written informed consent. From 39 patients (19 females, 20 males) who fulfilled diagnostic criteria of definite s-IBM frozen muscle tissue stored at −80 °C was available for further analysis. Age at biopsy and duration of disease did not differ between male and female patients. Clinically, a typical proximal and distal asymmetric limb weakness was present in 34 patients, while 4 patients manifested with purely proximal and 1 patient with purely distal motor deficits at the time of biopsy; 21 patients developed dysphagia during the course of the disease. Eleven patients had received a treatment with either low-dose steroids alone ($n = 5$) or combined with azathrioprine or methotrexate ($n = 6$) before biopsy. The definitive diagnosis of s-IBM was based on established clinico-pathological criteria [5,22] and sarcoplasmic immunoreactivity for b-amyloid, phosphorylated tau, p62 [23] or TDP-43 protein [24] within >1% of muscle fibers. For comparison with other inflammatory myopathies, muscle biopsies from patients referred to the Reference Center for Neuromuscular Diseases, Institut de Myologie, Hôpital Pitié-Salpetrière who displayed treatment-responsive

Table 1
Demographic data of patients.

Disease	*n*	Median age at biopsy	Range	F/M	Median duration	Range
sIBM*	39	67 y	40–89 y	19/20	54 m	6–198 m
PM	13	43 y	16–72 y	11/2	5 m	1–108 m
DM	13	44 y	17–79 y	9/4	4 m	1–8 m
MD	10	43 y	26–77 y	7/3		
Co	10	64 y	55–74 y	5/5		
Total	85					

n = number of patients, y = years, m = months.
* Eleven patients with sIBM received a treatment with low-dose steroids ($n = 5$) or steroids combined with azathrioprine or methotrexate ($n = 6$) before biopsy. PM and DM patients had proximal limb weakness of less than 6 months duration with improvement after steroids, muscle biopsies fulfilled criteria of Dalakas and Hohlfeld [25].