Available online at www.sciencedirect.com
ScienceDirect
Neuromuscular Disorders 21 (2011) 345–352
www.elsevier.com/locate/nmd

Myonuclear breakdown in sporadic inclusion body myositis is accompanied by DNA double strand breaks

Makoto Nishii [a], Satoshi Nakano [a,b,*], Seika Nakamura [a], Reika Wate [a], Akiyo Shinde [a], Satoshi Kaneko [a], Hirofumi Kusaka [a]

[a] Department of Neurology and Brain Medical Research Center, Kansai Medical University, Japan
[b] Department of Neurology, Osaka City General Hospital, Japan

Received 29 September 2010; received in revised form 18 January 2011; accepted 2 February 2011

Abstract

Rimmed vacuoles in sporadic inclusion body myositis (s-IBM) contain nuclear remnants. We sought to determine if the nuclear degeneration seen in s-IBM is associated with DNA damage. In muscle biopsy specimens from ten patients with s-IBM and 50 controls, we immunolocalized 1) phosphorylated histone H2AX (γ-H2AX), which is a sensitive immunocytochemical marker of DNA double-strand breaks and 2) DNA-PK, which is an enzyme involved in double-strand break repair. In s-IBM, vacuolar peripheries often showed strong immunoreactivity to γ-H2AX and the three components of DNA-PK (DNA-PKcs, Ku70, and Ku80). A triple fluorescence study of Ku70, emerin, and DNA displayed nuclear breakdown and it suggested impaired nuclear incorporation of Ku70. The percentage of positive nuclei for γ-H2AX was significantly higher in vacuolated fibers than non-vacuolated fibers in s-IBM, or fibers in polymyositis. We hypothesize that a dysfunction of nuclear envelope may cause nuclear fragility, double-strand breaks and impaired nuclear transport in s-IBM.

Keywords: Inclusion body myositis; Rimmed vacuole; DNA double strand breaks (DSB); Nuclear breakdown

1. Introduction

Sporadic inclusion body myositis (s-IBM) is the primary cause of acquired myopathy in patients over 50-years old, but no effective therapy has yet been found [1,2]. The histopathological hallmarks of s-IBM consist of mononuclear cell infiltration, muscle fibers with congophilic inclusions, and rimmed vacuoles. Several studies showed nuclear components in the rimmed vacuoles (e.g., a single-stranded DNA binding protein of nuclear origin [3], emerin [4,5], lamin A/C [4] and histone H1 with DNA [5]). The findings indicate that the vacuoles may result from nuclear breakdown.

Terminally differentiated cells do not possess a replication-associated DNA repair mechanism, making them particularly sensitive to DNA damage [6]. Mature muscle cells are such terminally differentiated cells. In a muscle cell culture study, the exposure of differentiated myocytes to hydrogen peroxide resulted in the accumulation of foci of phosphorylated histone H2AX (γ-H2AX) [7], which is a sensitive marker of a serious form of DNA damage, DNA double strand breaks (DSB) [8]. DSB are produced by reactive oxygen species (ROS), ionizing radiation, and other genotoxic agents. Histone H2AX, a variant of histone H2A, is rapidly phosphorylated at Ser 139 in the chromatin region surrounding a DSB [9]. Immunocytochemical staining of γ-H2AX has been broadly applied to reveal DNA damage caused by cancer and other cellular stresses [8,10]. DNA-PK is an enzyme involved in the initial step of

* Corresponding author. Address: Department of Neurology, Osaka City General Hospital, 2-13-22 Miyakojima hondori, Miyakojima-ku, Osaka 534-0021, Japan. Tel.: +81 66929 1221, fax: +81 66929 1091.
E-mail addresses: s-nakano@hospital.city.osaka.jp, nakanos@takii.kmu.ac.jp (S. Nakano).

0960-8966/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.nmd.2011.02.004

the DSB repair process, non-homologous end joining (NHEJ), which does not require DNA replication, and therefore NHEJ is the predominant DNA repair mechanism in terminally differentiated cells [11,12]. DNA-PK consists of a catalytic subunit (DNA-PKcs) and two regulatory subunits (Ku70 and Ku80). The binding of heteroduplexes of Ku70 and Ku80 to DSB sites initiates the repair process [13,14]. In the current paper, we examine whether DSB are associated with myonuclear breakdown in s-IBM.

Note that DSB is different from the apoptotic DNA fragmentation that has been scarcely detected in the s-IBM muscles [15]. In DSB, DNA breaks occur directly and randomly by radiation or other genotoxic agents, whereas apoptotic DNA fragmentation takes place at a late stage of programmed cell death, in which endonucleases sever DNA strands at regular lengths. Apoptotic DNA fragmentation is not subject of repair or is not usually labeled with anti-γ-H2AX antibody.

2. Materials and methods

2.1. Patients

We studied muscle biopsy specimens from 10 patients (58–82 years old, 8 men and 2 women) who fulfilled the clinical, electromyographic, and histopathological criteria for s-IBM [16]. The muscle sections displayed cell infiltration surrounding non-necrotic fibers, congophilic inclusions and rimmed vacuoles in each patient. All s-IBM patients showed slowly progressive muscular symptoms (disease duration: 3.8 ± 2.9 years; mean ± standard deviation [SD], range: 0.5–9 years). None of these patients had received immunotherapy before the muscle biopsy. Specimens from five patients without pathologic alterations served as non-pathologic controls. For controls of other neuromuscular diseases, we used 45 muscle biopsies from patients with polymyositis ($n = 10$), dermatomyositis (8), dystrophinopathy (3), dysferlinopathy (3), mitochondrial encephalomyopathy (5), myotonic dystrophy type I (1), neurogenic muscular atrophy (5), oculopharyngeal muscular dystrophy (5), myopathy with autophagic vacuoles of an undetermined etiology (1), rhabdomyolysis (1), hypokalemic vacuolar myopathy (2), and colchicine myopathy (1). The above diagnoses were based on a clinical examination, family history, electromyography, and muscle biopsy studies. Polymyositis and dermatomyositis were diagnosed using conventional criteria [17]. The polymyositis sections contained several to many non-necrotic fibers surrounded by mononuclear cells, and the dermatomyositis sections demonstrated perifascicular atrophy or perimysial infiltration of inflammatory cells. This study was performed with the compliance of the internal review board of our institution.

2.2. Immunohistochemistry

Table 1 shows the primary antibodies applied and their concentrations. Immunohistochemical studies were performed as previously described [18]. Briefly, sections were fixed in cold acetone and then in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) before being blocked and incubated overnight at 4 °C with the primary antibody. The sections were then incubated with a biotin-labeled secondary antibody and developed using the avidin–biotin complex (ABC) immunoperoxidase method (Vector Laboratories, Burlingame, CA) with 3,3′-diaminobenzidine as the coloring agent. Next, the slides were lightly counterstained with hematoxylin for the quantitation of positive nuclei. The control experiments involved the omission of the primary antibody or the substitution of the primary antibody for non-immune mouse or rabbit IgG. We immunostained 12 or more sections from different individuals at a time, and the duration of color development was fixed. The specificity of antibodies for γ-H2AX and Ku70 was also tested in immunoblotting.

For triple-color immunofluorescence studies, the sections were incubated with anti-Ku70 plus anti-emerin antibodies followed by incubation with appropriate secondary antibodies for triple fluorescence (Chemicon International, Temecula, CA). The slides were mounted with Vectashield (Vector) containing 1.5 μg/mL of the nuclear DNA marker 4′,6-diamidino-2-phenylindole (DAPI) and examined with confocal imaging using the LSM510-META system (Carl Zeiss, Jena, Germany). As controls, we performed a single-color fluorescence study using each antibody or DAPI alone and confirmed the specificity of the secondary antibodies and filters.

Table 1
List of primary antibodies.

Antigen	Type	Clone/ID	Source	Concentration
γ-H2AX	MMA	JBW301	Upstate	1 μg/mL
Ku70	MMA	4C2–1A6	Abnova	1 μg/mL
Ku80	MMA	111	Abcam	1:500
DNA-PKcs	RPA	PC127	Calbiochem	5 μg/mL
Emerin	RPA	FL-254	Santa-Cruz Biotec	1 μg/mL
HNE	MMA	HNEJ-2	JaICA	20 μg/mL
iNOS	RPA	sc-651	Santa-Cruz Biotec	4 μg/mL
LAMP-2	MMA	H4B4	Santa-Cruz Biotec	4 μg/mL

MMA: mouse monoclonal antibody; RPA: rabbit polyclonal antibody.