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Histone H1 is released from myonuclei and present in rimmed vacuoles with DNA in inclusion body myositis

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Abstract

To investigate myonuclear alterations in sporadic inclusion body myositis (s-IBM), we immuno-localized histones in muscles in 11 patients. The examination showed that vacuolar rims were frequently positive for histone H1. In triple-color fluorescence study, the H1-positive products were found on the inner side of an emerin-positive circle with DNA. Moreover, H1-positive materials appeared to be released into the cytoplasm in some vacuoles and myonuclei. The localization of H1 was different from phosphorylated Elk-1, which is a nuclear protein, but abnormally accumulated in the cytoplasm in s-IBM. The results strongly support the hypothesis that rimmed vacuoles are derived from the nucleus. The cytoplasmic H1-release suggests dysfunction of nuclear membranes in an early phase of the nuclear disintegration. We hypothesize that, in s-IBM muscles, compromised nuclear envelope may permit release of some nuclear components such as histone H1 and cannot facilitate the incorporation of others to the nucleus as in pElk-1.

Keywords: Inclusion body myositis; Distal myopathy; Rimmed vacuole; Nuclear degeneration; Immunohistochemistry; Pathology

1. Introduction

Sporadic inclusion body myositis (s-IBM) is a main cause of muscular disability in people over 50 years old and is refractory to therapies. The diagnostic criteria of s-IBM on muscle biopsy are (1) inflammatory exudates surrounding non-necrotic fibers, (2) rimmed vacuoles, which are rounded or irregularly polygonal spaces in fibers with basophilic granules, and (3) congophilic inclusions in light microscopy or filaments that are 15–20 nm in diameter in electron microscopy [1,2]. In addition to these pathological findings, several studies showed distinct myonuclear alterations in s-IBM. Firstly, under electron microscopy, filamentous inclusions were sometimes detected in myonuclei as well as in the cytoplasm. Rarely, these inclusions appeared to be released from nuclei into the cytoplasm with breaks in the nuclear membrane [1]. Secondly, a study showed abnormal expression of single-stranded DNA binding protein of nuclear origin in muscle cytoplasm [3]. Thirdly, perinuclear localizations of nuclear transcription factor and nucleus-oriented protein kinases suggested inhibition of nucleocytoplasmic transport [4,5]. Two groups showed that rimmed vacuoles might originate from the breakdown of the nucleus [3,6], although most of the studies hypothesized that rimmed vacuoles are autophagic and composed of lysosomes [7,8]. If rimmed vacuoles are of nuclear origin, the basophilic materials in rimmed vacuoles should be components of the nucleus.

Histones represent basophilic nuclear proteins. In inactive chromatin, the DNA is complexed to histones and forms nucleosomes. A nucleosome is an octamer of four pairs of the core histones H2A, H2B, H3, and H4, around which double-stranded DNA is wound. Histone H1 binds to the linker DNA that connects the individual nucleosomes. It is essential for the generation of the highly condensed chromatin structure and plays a pivotal role in gene regulation [9]. H1 is rich in arginine and lysine residues, which makes it highly basic [10].

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In the current study, we examined histones in s-IBM by immunohistochemistry. We show that H1-positive products often delineated vacuolar boundaries in s-IBM. In addition, we detected H1 beyond intact nuclear membranes, which suggests release of nuclear components. The localization of H1 was usually different from that of phosphorylated Elk-1 (pElk-1), which is a nuclear protein, but shows extranuclear localization in abnormal fibers in IBM [5].

2. Materials and methods

2.1. Patients

We investigated muscle biopsy specimens from 11 patients (56–82 years old, 68.18 ± 7.44: mean $\pm$ SD; 9 men and 2 women) who fulfilled the diagnostic criteria of s-IBM [11]. Each muscle specimen contained congophilic inclusions, rimmed vacuoles, and inflammatory exudates. All s-IBM patients showed slowly progressive muscular disability (disease duration 3.73 ± 2.71 years; mean $\pm$ SD). No patients received corticosteroid or immunosuppressants before biopsy. Muscle specimens deemed free from pathologic alterations from three patients served as normal controls. For disease controls, we used 35 muscle biopsies from patients with polymyositis ($n = 12$), dermatomyositis (6), dystrophinopathy (2), dysferlinopathy (1), myotonic dystrophy type I (1), neurogenic muscular atrophy (4), oculopharyngeal muscular dystrophy (3), distal myopathy with rimmed vacuoles (3), acid maltase deficiency (1), myopathy with autophagic vacuoles with undetermined etiology (1), and hypokalemic vacuolar myopathy (1). The diagnoses were based on the clinical examination, family history, EMG, and muscle biopsy studies.

2.2. Primary antibodies

The following primary antibodies were used at concentrations described: mouse monoclonal anti-histone H1 (clone AE-4) at 0.5 μg/mL (Santa Cruz Biotechnology, Santa Cruz, CA); mouse monoclonal anti-histone H1 (clone 145-1) at 2 μg/mL (LAB VISION Co., Fremont, CA); rabbit polyclonal anti-histone H2A at 2 μg/mL (H-124, Santa Cruz); rabbit polyclonal anti-histone H2B at 1:200 dilutions (AB1623, Chemicon International, Temecula, CA); rabbit polyclonal anti-histone H3 at 2 μg/mL (FL-136, Santa Cruz); rabbit polyclonal anti-histone H4 at 2 μg/mL (ab10158, Abcam, Cambridge, UK); mouse monoclonal anti-emerin (clone 4G5) at 1:20 (Novocastra Laboratory, New castle upon Tyne, UK); rabbit polyclonal anti-emerin (FL-254, Santa Cruz) at 1 μg/mL; rabbit polyclonal anti-Elk-1 phosphorylated at Ser383 at 1:75 (#9181, Cell Signaling Technology, Danvers, MA).

2.3. Immunohistochemical studies

Transverse cryostat sections (7 μm) were used for immunohistochemical studies. Sections were fixed in cold acetone and then in 4% paraformaldehyde in 0.1 M phosphate-buffer for 10 min. The treatment with paraformaldehyde was necessary to fix DNA properly. After washing, non-specific binding was blocked by preincubation in phosphate-buffered saline (PBS), pH 7.4, containing 2% bovine serum albumin and 5% normal serum of animals from which the secondary antibody was raised. The sections were then incubated overnight at 4 °C in blocking solution containing the primary antibody, followed by incubation with a biotin-labeled secondary antibody (Vector, Burlingame, CA). The sections were then developed using the avidin–biotin complex (ABC) immunoperoxidase method (Vectastain ABC kit, Vector). The immunostained sections were counter-stained lightly with eosin to identify vacuoles. Control experiments included the omission of the primary antibody and the substitution of the primary antibody with non-immune mouse or rabbit IgG.

For triple-color immunofluorescence studies, the sections were incubated with (1) anti-H1 plus anti-emerin (FL-254) antibodies, (2) anti-H1 plus anti-pElk-1 antibodies, or (3) anti-emerin (4G5) plus anti-pElk-1 antibodies at 4 °C overnight, followed by incubation with secondary antibodies, consisting of rhodamine-labeled donkey anti-mouse IgG (AP192R, Chemicon; 1:50 dilutions) and FITC-conjugated donkey anti-rabbit IgG (AP182F, Chemicon; 1:50), both of which are compatible with dual fluorescence. The slides were mounted with Vectashield (Vector) containing 1.5 μg/mL of a nuclear DNA marker 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI), and viewed with an Olympus photomicroscope (Tokyo, Japan) equipped for epifluorescence. Images were acquired with a PXL1400 cooled CCD camera (Photometrics, Huntington Beach, AZ) controlled by software (Scanalytics, Fairfax, VA). After obtaining micrographs, some of the sections were stained with H&E and compared with the results in immunofluorescence studies. For controls, we performed a single-color fluorescence study using each antibody or DAPI alone and confirmed specificity of the secondary antibodies and color-filters.

For calculating percentages of fibers containing vacuoles rimmed by histone H1, pElk-1, or histone H1 plus pElk-1, we photographed more than 10 pictures per section stained with immunoperoxidase method in each s-IBM patient. We did not count immunopositive deposits as rims when they looked like normal nuclei in vacuoles.

3. Results

3.1. Expression of histone H1 in s-IBM

A proportion of fibers harbored one or more immunopositive rings of H1 in s-IBM (Fig. 1). With eosin counter-staining, the inside spaces were lucent. Therefore, the positive rings corresponded to the vacuolar boundaries. Some vacuoles contained H1-positive closed rings, while others harbored broken circle or several dots of H1-positive immunoreaction. The table shows the relative percentage