Neuroscience Letters 638 (2017) 55–59

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

The origins of rimmed vacuoles and granulovacuolar degeneration bodies are associated with the Wnt signaling pathway

Yukari Murata-Shinozaki[a], Tetsuya Takahashi[a,*], Tomoyasu Matsubara[a], Hirofumi Maruyama[a], Yuishin Izumi[b], Masayasu Matsumoto[a,c]

[a] Department of Clinical Neuroscience and Therapeutics, Hiroshima University Graduate School of Biomedical and Health Sciences, 1-2-3 Kasumi, Minami-ku, 734-8551 Hiroshima, Japan
[b] Department of Clinical Neuroscience, Institute of Health Biosciences, Graduate School of Medicine, University of Tokushima, Tokushima, Japan
[c] JCHO, Hoshigaoka Medical Center, Hirakata, Osaka, Japan

HIGHLIGHTS

- RVs and GVD bodies share a number of molecules.
- Wnt signaling might be involved in the formation of RVs and GVD bodies.
- RV and GVD body formation share common pathogenic mechanisms.

ARTICLE INFO

Article history:
Received 12 October 2016
Received in revised form
29 November 2016
Accepted 6 December 2016
Available online 7 December 2016

Keywords:
Alzheimer's disease
Granulovacuolar degeneration
Inclusion-body myositis
Rimmed vacuole
Wnt

ABSTRACT

Inclusion-body myositis (IBM) and Alzheimer's disease (AD) are biochemically characterized by the presence of aggregated β-amyloid protein and tau protein. In addition, both diseases are pathologically characterized by vacuolar changes, including rimmed vacuoles (RVs) in IBM and granulovacuolar degeneration (GVD) in AD. Previously, we demonstrated that RVs and GVD bodies are associated with a set of common molecules, leading us to speculate that both RVs and GVD bodies originate from similar structures on the plasma membrane of muscle cells and neuronal cells, namely, the neuromuscular junction (NMJ) and the postsynaptic spine especially in terms of Wnt signaling pathway. In this study, we investigated the presence of components of NMJ in RVs and/or postsynaptic spine in GVD bodies respectively by immunohistochemistry and immunofluorescence. The antigens probed included the following: (1) dishevelled (Dvl) family proteins (Dvl1, Dvl2 and Dvl3), (2) NMJ-associated proteins (low density lipoprotein-related protein 4 [Lrp4], heat shock protein 70 [Hsp70], β-catenin, phospho-β-catenin, rapsyn, P21-activated kinase 1 [PAK1], adenomatous polyposis coli [APC] and ADP-ribosylation factor 6 [Arf6]), (3) a lipid raft-associated molecule (phosphatidylinositol 4, 5-bisphosphate [PIP2]), and (4) other proteins [prion, glycogen-synthase kinase 3β (GSK-3β)]. In all cases of sporadic IBM examined, RVs were immunopositive for Dvl3, Hsp70, β-catenin, PIP2, APC, prion and GSK-3β. In all cases of AD examined, GVD bodies were immunopositive for Dvl3, phospho-β-catenin, rapsyn, APC and PIP2. These findings show that RVs and GVD bodies share common molecules associated with the Wnt signaling pathway, indicating that these structures share a common structural and functional origin.

1. Introduction

Rimmed vacuoles (RVs) are found in a number of muscular disorders, including inclusion-body myositis (IBM) [1], distal myopathy with RV formation, oculopharyngeal dystrophy [2], and Becker muscular dystrophy [3]. RVs are approximately 3–20 μm in diameter and consist of vacuoles surrounded by filamentous material forming round/oval or cleft-like shapes [1]. Most vacuoles are empty, but are sometimes occupied by granules. Sporadic IBM (s-

Abbreviations: AD, Alzheimer's disease; APC, adenomatous polyposis coli; Arf6, ADP-ribosylation factor 6; CK1δ, casein kinase 1δ; Dvl, dishevelled; GSK-3β, glycogen-synthase kinase 3β; GVD, granulovacuolar degeneration; Hsp70, heat shock protein 70; Lrp4, low density lipoprotein-related protein 4; PAK1, P21-activated kinase 1; p-β-catenin, phospho-β-catenin; PIP2, phosphatidylinositol 4, 5-bisphosphate; PSD95, postsynaptic density protein 95; RV, rimmed vacuole; s-IBM, sporadic inclusion body myositis.

* Corresponding author.
E-mail address: tetakaha@hiroshima-u.ac.jp (T. Takahashi).

http://dx.doi.org/10.1016/j.neulet.2016.12.014
0304-3940/

IBM) is one of the most common muscle diseases, with prominent RVs in patients over the age of 50 [4]. The pathology of s-IBM is similar to that of Alzheimer's disease (AD), in that both are associated with β-amyloid (Aβ) peptide and phosphorylated tau [5].

AD is characterized pathologically by the presence of senile plaques and neurofibrillary tangles. Furthermore, granulovacuolar degeneration (GVD) in the brain is also a pathological hallmark of AD [6]. GVD bodies, which are typically 3–5 μm in diameter, are accumulations of basophilic small granules, each within a clear vacuole, in the perinuclear region of pyramidal neurons [7]. GVD bodies contain many proteins related to tau phosphorylation, including casein kinase 1 (CK1 [CK1δ and CK1ε]) [8] and glycogen-synthase kinase 3β (GSK-3β) [9]. Earlier, we reported that GVD bodies contain another tau kinase, cyclin dependent kinase 5 (CDK5) [10]. In addition, we demonstrated that the lipid raft-associated molecules, flotillin-1, phosphatidylinositol 4, 5-bisphosphate (PIP2) and annexin 2 are present in GVD bodies [11]. Membrane lipid rafts are small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes [12] and those in postsynaptic spines are regarded as major sites of signal transduction, membrane trafficking and molecular sorting [13,14].

Previously, we demonstrated that GVD bodies in neurons and RVs in muscle cells contain a number of common proteins, including charged multivesicular body protein 2 B (CHMP2B), caspase 3, flotillin-1, annexin 2, leucine-rich repeat kinase 2 (LRRK2), CDK5 and CK1δ [15]. CHMP2B, a subunit of the endosomal sorting complex required for transport (ESCRT) complex-III, is important for the formation of multivesicular bodies and subsequent late endosomes [16]. The presence of CHMP2 B in both RVs and GVD bodies [15,17], indicates that plasma membranes might be endocytosed to form these structures. Provided that raft domains on the plasma membrane were endocytosed and formed RVs in muscle cells and GVD bodies in neurons, the origins of these structures might be similar functionally and structurally in both of these cell types.

In the present study, as such candidate lipid rafts structures we focused on both neuromuscular junctions (NMJ) in the muscle for RVs and postsynaptic spine in the brain for GVD bodies. We aimed to examine the biochemical similarities between RVs and GVD bodies by immunolabeling using antibodies for molecules that are present not only in lipid rafts but also in the NMJ and the postsynaptic spine.

2. Materials and methods

We performed immunohistochemical and immunofluorescent staining procedure as previously described [15]. Ethics statement and detailed processes are described in Supplementary data.

3. Results

3.1. RVs are immunopositive for molecules located in the NMJ

In all cases of s-IBM, RVs were immunopositive for Dvl3, Hsp70, β-catenin, APC, PIP2, prion and GSK-3β (Fig. 1). Rapsyn was detected in RVs of six, but not all, s-IBM cases (Fig. 1). β-catenin, PIP2 and GSK-3β were also present in some nuclei. Dvl3 and rapsyn were diffusely distributed in the cytoplasm of RV-positive fibers, as well as on RVs. In comparison, Dvl1, Dvl2, Lrp4, phospho-β-catenin, PAK1 and Arf6 were not detected in RVs of any s-IBM cases (data not shown). The cytoplasmic membranes, but not RVs, were positive for Lrp4 in all s-IBM cases (data not shown). A summary of immunostaining results is shown in Table 1.

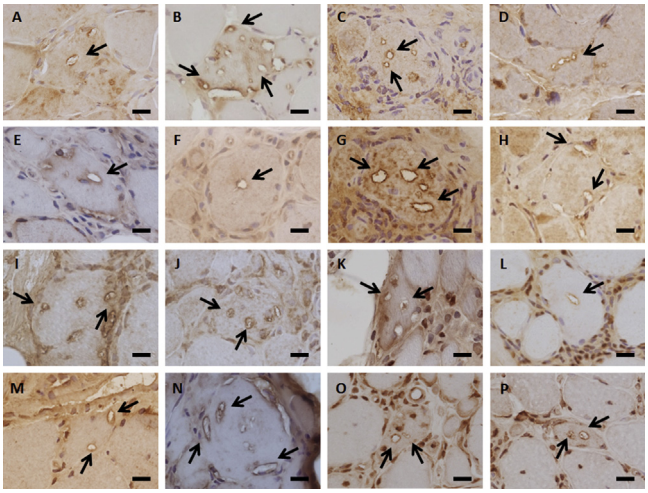

Fig. 1. Immunohistochemistry in s-IBM muscle. Dvl3 (A, B), Hsp70 (C, D), β-catenin (E, F), rapsyn (G, H), APC (I, J), PIP2 (K, L), prion (M, N) and GSK-3β (O, P) are detected (positive labeling) in RVs in s-IBM. Arrows indicate RVs. Scale bars = 20 μm.

Table 1
Summary of immunostaining results.

	RV	GVD
Dvl1	–	–
Dvl2	–	–
Dvl3	+	+
Lrp4	–	–
Hsp70	+	–
β-catenin	+	–
p-β-catenin	–	+
rapsyn	+	+
PAK1	–	–
APC	+	+
Arf6	–	–
PIP2	+	+
prion	+	–
GSK3-β	+	N/A

N/A, not assessed.

3.2. GVD bodies are immunopositive for molecules located in the NMJ

In GVD bodies, Dvl3, phospho-β-catenin, rapsyn, APC and PIP2 were detected (Fig. 2). In addition, the signals for these antigens were diffusely detected in the cytoplasm of pyramidal cells. Dvl1, Dvl2, Lrp4, Hsp70, β-catenin, PAK1, Arf6 and prion were not present in GVD bodies (data not shown). Dvl1, Dvl2, Lrp4, Hsp70, PAK1, Arf6 and prion were diffusely detected in the cytoplasm of pyramidal cells, while β-catenin was not detected in these cells (data not shown). We also examined immunoreactivity for synaptophysin, a presynaptic marker, and postsynaptic density protein 95 (PSD95), a postsynaptic marker, to test whether GVD bodies contain presynaptic or postsynaptic components. Neither synaptophysin nor PSD95 were detected in GVD bodies (data not shown). A summary of immunostaining results is shown in Table 1.

3.3. pTDP43 or CHMP2B colocalizes with all of the antigens present in RVs and CK1δ colocalizes with all of the antigens present in GVD bodies

We performed double immunofluorescence staining using anti-pTDP43 or anti-CHMP2B antibody (markers of RVs) and anti-CK1δ (a marker of GVD bodies) along with antibodies to the antigens detected in RVs and/or GVD bodies. pTDP43 or CHMP2B colocalized with all of the antigens detected in RVs, and CK1δ colocalized with