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Sporadic Inclusion Body Myositis: Possible pathogenesis inferred from biomarkers

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Abstract

Purpose of review—The relevance of proteins that accumulate and aggregate in the muscle fibers of patients with sporadic inclusion body myositis (sIBM) is unknown. Many of these proteins also aggregate in other disorders, including Alzheimer’s disease, leading to speculation that sIBM pathogenesis has similarities to neurodegenerative disorders. Our review will discuss current studies on these protein biomarkers and any utility in sIBM diagnosis.

Recent findings—Two “classical” components of sIBM aggregates (A β and phospho-tau) have been re-evaluated. Three additional components of aggregates (TDP-43, p62, and LC3) have been identified. The sensitivity and specificity of these biomarkers has been explored. Two studies suggest that TDP-43 may have clinical utility in distinguishing sIBM from other inflammatory myopathies.

Summary—The fact that sIBM muscle accumulates multiple protein aggregates with no single protein appearing in every sIBM patient biopsy suggests that it is not presently possible to place pathogenic blame on any single protein (i.e. A β or TDP-43). Instead changes in protein homeostasis may lead to the accumulation of different proteins that have a propensity to aggregate in skeletal muscle. Therapies aimed at improving protein homeostasis, instead of targeting a specific protein that may or may not accumulate in all sIBM patients, could be useful future strategies for this devastating and enigmatic disorder.

Keywords

inclusion body myositis; TARDNA binding protein-43; biomarkers; inflammatory myopathies; proteostasis

Introduction

Sporadic inclusion body myositis (sIBM) is an idiopathic and untreatable myopathy that typically begins in patients over the age of 50 (1). Patients have a characteristic pattern of involvement with both proximal and distal muscle weakness and a predilection for the knee extensors and wrist and finger flexors. Disease progression leads to significant morbidity with wheelchair confinement often within 10 years of onset (1). Some clues to the pathogenesis of sIBM have come from its muscle pathology. This review will discuss possible pathogenic factors in sIBM in relation to diagnostic tissue markers found in sIBM muscle biopsies.

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sIBM muscle pathology

Biopsies from patients with sIBM can have several pathologic features that aid in distinguishing sIBM from other inflammatory and inherited muscle disorders. These include: 1) *Endomysial T-cell infiltrates* that surround healthy-appearing muscle fibers in most sIBM biopsies. This is accompanied by “*focal invasion*” in which normal-appearing muscle fibers are invaded by CD8+ T cells (2–4). 2) The *major histocompatibility complex class I* is upregulated on most muscle fibers in sIBM, even fibers that appear histologically normal (5). 3) *Vacuoles*, classically described as “rimmed”, are present in scattered non-necrotic fibers. These vacuoles do not contain any specific protein nor are they lined by any single protein making their formation and presence particularly enigmatic (6, 7). 4) *Congophilic amyloid* staining has been suggested to be a marker of sIBM (8). Congophilic amyloid is often detected via apple green-red birefringence under polarizing light. Congo red staining viewed with indirect immunofluorescence may increase sensitivity of detecting aggregates in muscle fibers in sIBM (9). Caution is required when calling these “fluorescent” structures amyloid since Congophilic amyloid is defined by its ability to bind and intercalate Congo red dye creating the birefringence. 5) “*Sarcoplasmic inclusions*” or “*Protein aggregates*” are composed of several proteins that have received great attention because some, such as amyloid beta (A β), are associated with neurodegenerative disorders (10). The specificity of these protein aggregates for sIBM remains debated since some patients with sIBM-like syndromes have no protein aggregates in muscle fibers (11–15). 6) *Mitochondrial DNA deletions* occur in scattered myofibers of a majority of sIBM patients (16, 17). These are detected by an increase in succinate dehydrogenase (SDH) staining and a decrease in cytochrome oxidase (COX) staining. Mitochondrial pathology occurs in IBM-like inflammatory myopathies that have no vacuoles, protein aggregates or Congophilic inclusions (18).

Biomarkers: Old, new and revisited

As noted, multiple proteins can accumulate in sIBM patient muscle (Figure 1). Most of these proteins were selected for study based upon their propensity to aggregate and/or abnormalities in other muscle or neurodegenerative disorders. The diagnostic, and especially pathogenic, significance of many of these proposed biomarkers has not been well established. Several recent studies have examined such questions.

Beta amyloid (A β) fragments

The accumulation of the A β fragment of amyloid precursor protein (APP) in brain is the defining feature that underlies the “amyloid hypothesis” of Alzheimer’s disease (AD) pathogenesis (19). APP is proteolytically processed at its C-terminal end into a 39–42 amino acid peptide, known as A β or A β 1–40/42 depending upon its length (20). The A β fragment is the principal component of extracellular plaques in AD (21). Familial AD mutations in APP or presenilin1/2 lead to an increase in the processing of APP and, more specifically, the secretion of the more aggregate prone A β 1–42 peptide (20). Congophilic amyloid aggregates and immunoreactivity to the A β region of the amyloid precursor protein (APP) have been found within a minority of skeletal muscle fibers in some patients with sIBM (14, 15). More recently, antibodies that bind to the C-terminus of the A β peptide but not the full length APP protein have demonstrated aggregates of the A β fragment, and the more amyloidogenic A β 1–42, in the muscle fiber cytoplasm, and associated with vacuoles, in sIBM (22). Sandwich ELISA assays detected A β 1–42, but no A β 1–40, in patient muscle homogenates when compared with 4 age matched control muscles (22). This is surprising since, under normal conditions, A β 1–40 is processed from APP at a rate 10 times that of A β 1–42 (20). Whether muscle processes A β differently than other cells remains to be established. Regardless, this data is the first step in identifying true A β fragments in skeletal muscle. Confirmation of the presence of A β fragments in sIBM muscle remains to be further

established via immunoblot. There is no data regarding the specificity or sensitivity for sIBM of A β fragment accumulation. For example, it is important to determine whether A β fragment specific antibodies see changes with the upregulation of APP seen in regenerating muscle fibers that can be distinguished from sIBM pathology (23).

Phosphorylated tau

The SMI-31 antibody was originally used to identify phosphorylated neurofilament heavy chains (13). SMI-31 has been shown to label protein aggregates in muscle fibers in sIBM, a pathology that may have some specificity for the disease (24). As neurofilaments are not generally present in muscle fibers, it has been proposed that SMI-31 cross reacts with other phosphorylated proteins, including tau, a component of neurofibrillary tangles (24). To evaluate the specificity, and identify the immunoreactive antigen, of several phospho-tau antibodies in normal and sIBM muscle, skeletal muscle was immunostained with 3 tau antibodies, two with proposed immunoreactivity to phospho-tau and one to non-phosphorylated tau (13). These antibodies localized to the nucleus of myofibers in control and sIBM patient biopsies (13). Immunoblot of sarcoplasmic and nuclear fractions from normal muscle with antibodies SMI-31 or pS422 (phospho-tau specific) identified multiple proteins outside of the molecular size range for tau (13). The study concluded that the true identity of the SMI-31 immunoreactive proteins in sIBM is uncertain (see section on p62 for a possible candidate).

TAR DNA binding protein – 43 (TDP-43)

Mutations in TDP-43 are associated with a familial form of amyotrophic lateral sclerosis (ALS) (25–27). TDP-43 is a major component of ubiquitinated inclusions in ALS and fronto-temporal lobar dementia (FTLD-U) (28). In these disorders, TDP-43 is modified by phosphorylation and ubiquitination (28), and its normal, predominantly nuclear, localization changes to the cytosol where it forms cytoplasmic inclusions (28). Five recent studies have examined TDP-43 inclusions in sIBM skeletal muscle fibers (14, 15, 29–31). In contrast to normal tissue which has predominantly nuclear TDP-43 immunostaining, sIBM muscle contains sarcoplasmic TDP-43 inclusions (14, 31). Similar to changes seen in ALS and FTLD-U brain, TDP-43 was absent from the nucleus of TDP-43 inclusion bearing myofibers (14, 31). Sarcoplasmic TDP-43 co-stained with ubiquitin and phospho-TDP-43 specific antibodies (30, 31). Similar TDP-43 inclusions were also identified in other disorders with “rimmed vacuoles” including, IBMPPD, HIBM2 and myofibrillar myopathies where it co-localized with desmin, myotilin and SMI-31 (14, 29–31). Immunoblots for TDP-43 using lysates from patient’s muscle with sIBM confirmed that TDP-43 is present and modified in sIBM patient tissue (31). The utility of TDP-43 as a biomarker is discussed below.

Autophagy Markers p62 and LC3

The two predominant proteolytic systems in skeletal muscle are autophagy and the ubiquitin proteasome system (UPS) (32). Macroautophagy involves non-selective degradation of cytoplasmic regions containing proteins and cellular organelles, including mitochondria (33). This contrasts with the UPS system which selectively degrades proteins marked with a ubiquitin tag (32). Autophagy may be important in the development of muscle fiber atrophy (34, 35). The initiation of autophagy can occur via several stimuli, most notably nutrient deprivation (36). Upon stimulation of autophagy, a region of cytoplasm is sequestered into double membrane bound lysosomes (37). Autophagosomes are visualized by immunohistochemistry using antibodies to the protein LC3 (38). Another commonly evaluated autophagic protein is p62 or sequestosome (39). Unmodified LC3 is a soluble protein (LC3I) (38). When a pre-autophagic structure is induced LC3I is cleaved into LC3II and becomes covalently linked to the growing membrane of an autophagosome (38). p62 binds to ubiquitinated proteins and LC3 (40). It serves as scaffold for delivery of

ubiquitinated proteins to the interior of the autophagosome for degradation (41). Both p62 and the LC3II isoform of LC3 are upregulated and degraded during autophagy making the interpretation of static levels of these proteins difficult (42).

An increase in LC3 positive autophagosomes in the sarcoplasm of muscle fibers is consistent with two contradictory hypotheses. 1) Autophagy has been induced resulting in the accumulation of autophagosomes, suggesting an intact functioning autophagic system, or 2) Autophagosomes are accumulating because they fail to be degraded (i.e. fuse with a lysosome), suggesting a failure in autophagy. Therefore, caution must be used when interpreting results from LC3 and p62 immunostaining (43). One study looking at p62 in sIBM muscle identified p62 immunoreactivity in both sIBM and some control muscles (44). p62 immunoreactivity was greatest in sIBM muscle and co-localized with SMI-31 and a phospho-tau antibody (44). SMI-31 may immunoreact against p62 from purified Mallory bodies in hepatocytes suggesting that the SMI-31 immunoreactivity seen in sIBM muscle may be to p62 (13, 45). Consistent with the pattern of immunostaining, p62 was increased three fold in sIBM tissue homogenates when compared to normal patients, a finding which has been confirmed by others (44, 46). The sensitivity and specificity of p62 was not evaluated. p62 staining was also seen in targets and small angular fibers in denervated muscle, and in scattered fibers from patients with polymyositis (44).

Another study evaluated LC3 staining in a large retrospective series of muscle biopsies, including 23 sIBM, 26 polymyositis with mitochondrial features (PM-Mito, an sIBM-like syndrome) and 12 steroid responsive polymyositis muscle biopsies (15). LC3 aggregates were present in 85% of sIBM, 87% of PM-Mito and 17% of polymyositis biopsies (15). They concluded that LC3 aggregates may help to distinguish between treatment responsive and treatment refractory polymyositis. Moreover, four patients with PM-Mito later developed sIBM on repeat biopsies, suggesting that upregulation of LC3 may be an early feature of sIBM muscle pathology (15).

LC3 immunostaining in sIBM may co-localize to muscle fibers with APP accumulations (47). The presence of LC3 positive fibers was reported as not being specific for sIBM since it was found in another vacuolar myopathy, acid maltase deficiency (47). LC3 aggregates are rare in control muscle.

Mouse studies have shown tissue specific knockout of autophagy essential proteins such as ATG5 and ATG7 can produce progressive weakness and atrophy (34, 35). In addition, their muscle accumulates ubiquitinated inclusions, p62 aggregates and damaged mitochondria (34). Inhibition of autophagosome maturation with drugs like chloroquine or with genetic mutations in autophagosome fusion proteins results in the accumulation of ubiquitinated proteins, p62 and LC3 positive autophagosomes (46, 48). In some cases these proteins localize to central regions of the muscle fiber in patterns similar to rimmed vacuoles (46). Impairing autophagosome-lysosome fusion via similar strategies in skeletal muscle results in TDP-43 pathology similar to that seen in sIBM (46).

Biomarker specificity

Biomarkers can be utilized as diagnostic tools when they are both sensitive and specific for a diagnosis. Non-specific diagnostic biomarkers may be more valuable when used in conjunction with other disease features. Biomarkers can also have prognostic implications or be used to monitor the disease course or even suggest pathogenesis, whether or not they are specific. Two recent studies have evaluated the percentage of pathologically and clinically defined patients with specific biomarkers as well the “burden” of pathologic protein accumulation in sIBM muscle (14, 15). In one paper, sIBM had more rapid progression of weakness than PM-Mito, which has fewer protein aggregates (15). The most sensitive

marker for sIBM was TDP-43 which was present in 77% of sIBM biopsies compared to only 17% and 8% of PM-Mito and steroid responsive polymyositis muscle biopsies respectively (15). Another study evaluated the number of fibers in each sIBM biopsy that contained sarcoplasmic TDP-43 (14). They retrospectively compared muscle from 23 patients with sIBM to 17 disease controls and 27 other inflammatory myopathies. In the 23 sIBM patients, sarcoplasmic TDP-43 was found in 25% of all myofibers, while rimmed vacuoles were found in only 2.8% and SMI-31 immunoreactivity in only 0.83% (14). TDP-43 is not only present in more patients with sIBM but is an abundant component of sIBM muscle.

Is it a fair to infer pathogenesis from tissue biomarkers?

The identification of Congophilic amyloid inclusions in sIBM skeletal muscle of patients has led to a proposal that sIBM has similar pathogenic features to neurodegenerative illnesses, including Alzheimer's disease (49). In addition to A γ fragments and SMI-31 reactivity of aggregates, other aggregate prone proteins have been anecdotally identified in sIBM muscle. These include α -synuclein, TDP-43 and prion protein (PrP) (31, 50, 51). Placing pathogenic responsibility on α -synuclein in Parkinson's disease, A β in Alzheimer's disease and TDP-43 in ALS is justified since these disorders accumulate the respective proteins with some specificity and mutations in these proteins correlate with similar familial syndromes. The same cannot be stated for sIBM. To date no single protein appears to aggregate or deposit in all cases of sIBM (14, 15). Moreover, mutations in aggregate prone proteins, such as APP have not been identified in sIBM. sIBM is more aptly described as a "*promiscuous proteinopathy*" since sIBM muscle fibers acquire accumulations of assorted proteins (52). The relevance of any single protein to the pathogenesis is not well understood. Accumulation of any single aggregate-prone protein could be protective or lead to muscle degeneration. Muscle specific over expression of proteins known to aggregate in some sIBM patient muscle biopsies such as, APP, gelsolin and PrP has been studied in transgenic mouse models (53–55). Expression of these proteins can lead to the accumulation of the transgenic protein in myofibers, weakness and muscle degeneration (53–55).

Autosomal dominant mutations in gelsolin cause familial amyloidosis with deposition of amyloid in most tissues including skeletal muscle. Moreover, gelsolin is increased in sIBM (56) and gelsolin inclusions have been found in sIBM patient muscle tissue (57). Transgenic mice that overexpress a secreted and amyloidogenic form of gelsolin only in skeletal muscle were generated (54). Congo red fluorescence was initially detected around endomysial capillaries at three months of age (54). By 9 months extensive Congo red immunofluorescence accumulated in the endomysium surrounding muscle fibers, and by 18 months within scattered myofibers. At these same ages (9 and 18 months) mice became weak and developed muscle histopathology that resembled sIBM. Myopathology included rimmed vacuole formation and the accumulation of APP, ubiquitin and A β 1–42 immunoreactive inclusions. This animal model highlights how the expression of an aggregate prone protein can lead to pathology that resembles sIBM. More importantly, it demonstrates how expression of one mutant protein at high levels can secondarily result in the accumulation of other aggregate prone proteins in muscle fibers.

sIBM: A disorder of proteostasis

The appropriate balance of protein synthesis and degradation within a cell is essential to maintain protein homeostasis or "proteostasis (58)." Disruptions in proteostasis include the synthesis of a misfolded protein, alterations in the availability or functionality of protein chaperones, environmental stresses such as protein oxidation, and changes in the degradative capacity of the cell (58). The model of proteostasis implies that small changes at any of these steps (i.e. protein synthesis, folding, post-translational modification, or degradation) can have drastic consequences for the cell; ultimately leading to degeneration (59). The

gelsolin animal model described above is an example of disrupted proteostasis leading to myofiber degeneration (54). In this model, the expression a mutant protein with a propensity to aggregate (gelsolin) led to the accumulation of other aggregate prone proteins, undegraded ubiquitinated inclusions, vacuolation and finally myofiber degeneration.

Some familial myopathies with pathologic similarities to sIBM are termed hereditary inclusion body myopathies (HIBM). One rare form of HIBM (IBMPFD or inclusion body myopathy associated with Paget's disease of the bone and fronto-temporal dementia) is due to mutations in the chaperone protein p97/VCP (60). p97/VCP facilitates the degradation of ubiquitinated proteins via the UPS (61). p97/VCP has an instrumental role in autophagy as well (46, 62). Mutations in p97/VCP that cause IBMPFD impair autophagosome maturation to an autolysosome (46, 62). Expression of mutant p97/VCP in skeletal muscle causes a myopathy with ubiquitinated protein aggregates, sarcoplasmic TDP-43 inclusions and vacuolation (46, 63). These two mouse models (gelsolin and p97/VCP) with IBM-like pathology demonstrate how the synthesis of an aggregate prone protein or the impaired degradation of proteins can lead to a similar pathologic state. These animal models highlight the difficulty in defining a clear pathogenic mechanism in sIBM.

Conclusion

sIBM remains an enigmatic myopathy. To date, inferences into the pathogenesis have been surmised from pathologic snapshots seen in skeletal muscle biopsies of affected patients. In addition to inflammation, rimmed vacuoles and amyloid inclusions, these studies have demonstrated multiple proteins that seem to accumulate in diseased tissue. However the specificity and sensitivity of these proteins is still unclear. It is conceivable that small alterations in protein homeostasis result in the pathologic features seen in sIBM. Variability in disruptions in skeletal muscle proteostasis may explain why some patients muscle biopsies accumulate specific proteins whereas other do not.

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Figure 1. sIBM biomarkers aggregated in muscle fibers

All samples are from sIBM patients seen at the Washington University Neuromuscular clinic. A) Immunostaining with an A β 1–40 fragment specific antibody (3D6). B) TDP-43 immunostaining (red) and nuclei (blue). Note normal nuclear TDP-43 immunostaining (magenta) in adjacent non-inclusion bearing myofibers (open arrows). C) p62 immunostaining (red) and nuclei (blue). D) LC3 immunostaining (brown) with hematoxylin counterstain. Vacuoles are denoted by and protein inclusions with closed arrows. Scale is 100 μ M. ★