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REVIEW

Inclusion-body myositis: muscle-fiber molecular pathology and possible pathogenic significance of its similarity to Alzheimer's and Parkinson's disease brains

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Abstract Sporadic inclusion-body myositis (s-IBM), the most common muscle disease of older persons, is of unknown cause and lacks successful treatment. Here we summarize diagnostic criteria and discuss our current understanding of the steps in the pathogenic cascade. While it is agreed that both degeneration and mononuclear-cell inflammation are components of the s-IBM pathology, how each relates to the pathogenesis remains unsettled. We suggest that the intra-muscle-fiber degenerative component plays the primary role, leading to muscle-fiber destruction and clinical weakness, since anti-inflammatory treatments are not of sustained benefit. We discuss possible treatment strategies aimed toward ameliorating a degenerative component, for example, lithium and resveratrol. Also discussed are the intriguing phenotypic similarities between s-IBM muscle fibers and the brains of Alzheimer and Parkinson's diseases, the most common neurodegenerative diseases associated with aging. Similarities include, in the respective tissues, cellular aging, mitochondrial abnormalities, oxidative and endoplasmic-reticulum stresses, proteasome inhibition and multiprotein aggregates.

Keywords Inclusion-body myositis · Amyloid-beta · Multiprotein aggregates · Muscle-fiber degeneration · Inflammation · Endoplasmic-reticulum stress · Alzheimer's disease · Parkinson's disease · Lithium · Resveratrol · Aging

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Introduction

Sporadic inclusion-body myositis (s-IBM) is the most common muscle disease of older persons, age 55-plus. Its course is relentlessly progressive and there is no successful treatment. The s-IBM molecular muscle-fiber phenotype is virtually unique for a muscle disease, in that the pathological abnormalities within muscle fibers bear a number of strong similarities to Alzheimer's disease (AD) and Parkinson's disease (PD) brains (details below). In our opinion, those abnormalities play crucial roles in the s-IBM pathogenesis; they likely contribute importantly to the muscle-fiber degeneration and atrophy, which ultimately are responsible for the progressive muscle-fiber destruction to cause the relentlessly progressive clinical weakness.

In this article we present our current views on possible mechanisms leading to the characteristic features of s-IBM muscle fibers. We also present our newest data relevant to our concept that s-IBM is an age-associated disorder. And, we discuss the possible relationship between the degenerative and inflammatory components in s-IBM muscle tissues.

Because s-IBM still remains greatly underdiagnosed, we first present a short summary of the pathological diagnostic criteria that we consider important.

Diagnostic criteria of the s-IBM muscle biopsy

Light-microscopic histochemistry and immunocytochemistry

To diagnose s-IBM, and to help distinguish it from polymyositis, we suggest that the following stainings be performed on 10 μm transverse sections of a fresh-frozen muscle biopsy: (1) Engel trichrome staining [36] (Fig. 1a, b),

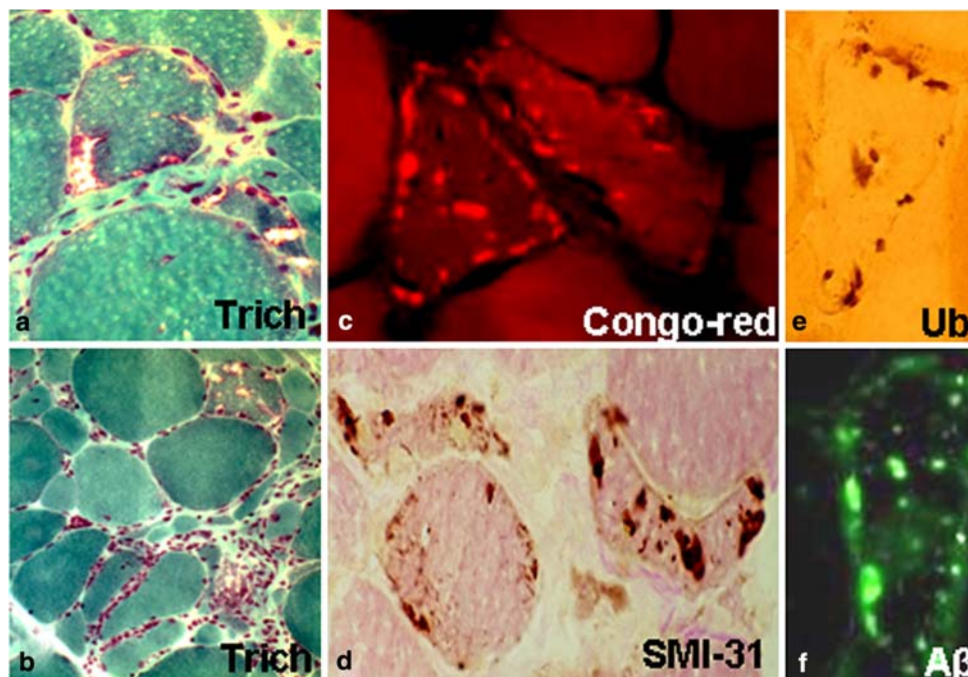

Fig. 1 Light-microscopic diagnostic features of the s-IBM muscle biopsy. **a**, **b** Engel Trichrome staining demonstrating vacuolated and atrophic muscle fibers, and mononuclear-cell inflammation. **c** Congo-red staining, visualized through Texas-red filters and epifluorescence illumination, shows various-sized amyloid deposits within two abnormal muscle fibers. **d** Diagnostic inclusions within muscle fibers identified by staining with SMI-31 antibody, which identifies phosphorylated tau. **e** Typical muscle-fiber inclusions identified with anti-ubiquitin antibody. **f** Various-sized amyloid-β immunoreactive inclusions within a muscle fiber. **a**, **b** ×1,250; **c–f** ×2,100

which reveals several to numerous muscle fibers containing one or a few *vacuoles* in a given section, and various degrees of lymphocytic inflammation (with some macrophages). While some of the vacuoles appear rimmed by a trichrome-reddish material (which indicates lipoprotein membranous material [36, 37]), often the vacuoles do not have a conspicuous reddish rim and appear "empty" (these must be distinguished from freeze-artefacts). (2) Fluorescence-enhanced Congo-red [6] to detect β-*pleated-sheet amyloid inclusions* (Fig. 1c). Multiple or single foci of amyloid, as identified by Congo-red fluorescence visualized through Texas-red filters [6], are evident within about 60–80% of the s-IBM vacuolated muscle fibers in a given transverse section, rarely within vacuoles but mostly in their non-vacuolated regions (the seemingly "amyloid-negative" fibers may have amyloid foci at other levels of those same fibers). This fluorescence-enhanced Congo-red technique is the best and most sensitive method for highlighting amyloid inclusions, which sometimes are very small or few. Crystal violet metachromasia staining can also show the intra-myofiber amyloid deposits [35], more conveniently but much less precisely. Congo-red visualized in polarized light, a widely used amyloid-seeking method, is the least precise and most difficult to interpret, and should not be used routinely for s-IBM muscle biopsies. (3) Staining with SMI-31 monoclonal antibody, which recognizes *phosphorylated tau (p-tau)*, in Alzheimer's disease (AD) brain and s-IBM muscle [3, 55, 67], and identifies *squiggly inclusions* containing *p-tau in* in s-IBM muscle fibers [3, 10] (Fig. 1d). If SMI-31 antibody is not available, *ubiquitin immunoreactivity* can be used to identify ubiquitin in both p-tau and Aβ intra-myofiber deposits of s-IBM (Fig. 1e) [16, 77]. Congo-red fluorescence, SMI-31 and ubiquitin immunoreactivities differentiate s-IBM from polymyositis, which does not have intra-fiber deposits positive with those reactions [10, 35]. (4) Immunostaining for amyloid-beta (Aβ) (Fig. 1f) is also useful but it is not required to diagnose s-IBM.

There are other light-microscopy aspects of s-IBM muscle biopsies that are characteristic and important, but not diagnostic for s-IBM. Examples are: (1) *mitochondrial abnormalities,* which include (a) *ragged-red fibers* [38], and (b) *cytochrome-c-oxidase (COX) negative muscle fibers* that are more common in s-IBM than expected for the patient's age [68, 79, and below]. (2) *Small angular muscle fibers,* which are histochemically dark with the pan-esterase and NADH-tetrazolium-reductase reactions. They are always present and are indistinguishable from those in ordinary denervation diseases. Those atrophic fibers are generally considered indicative of "*recent-denervation*" [37], and probably contribute significantly to the clinical weakness [35].

Ultrastructural abnormalities of s-IBM muscle fibers

Very characteristic are paired helical filaments (PHFs), often in clusters. They strikingly resemble PHFs of AD brain, being 15–21 nm diameter [7, 66]. They are present in both vacuolated and non-vacuolated muscle fibers (Fig. 2a, b) [detailed review in 10]. The s-IBM PHFs typically are immunostained with antibodies against phosphorylated tau by both gold- and peroxidase-immuno-electron microscopy (Fig. 2c, d).

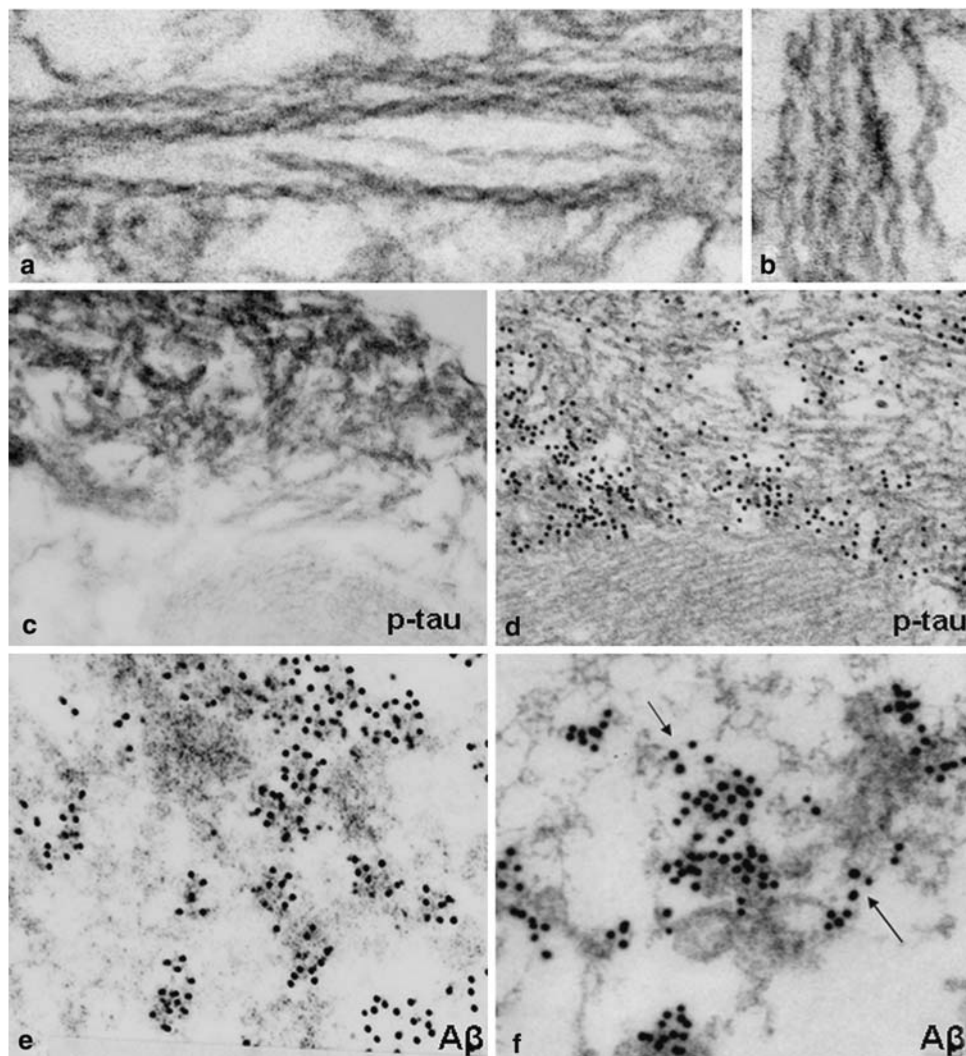

Fig. 2 Characteristic electron-microscopic abnormalities of s-IBM muscle fibers. **a**, **b** Several paired helical filaments (*PHFs*), in transmission electronmicroscopy. **c** Cluster of PHFs immuno-stained with AT8 antibody, which recognizes phosphorylated tau, and processed for horseradish-immunoperoxidase staining demonstrates dark reaction-product covering PHFs exclusively, while the adjacent portion of the myofiber is not immunostained. **d** Cluster of PHFs immuno-stained with SMI-31 antibody, which recognizes phosphorylated tau, and processed for gold-imuno-electronmicroscopy; this demonstrates that gold particles associate only with PHFs, while the adjacent portion of the myofiber is not immunostained. **e**, **f** Gold-immuno-electronmicroscopy of Aβ illustrates its localization on amorphous and floccular material, and on thin 6–10 nm amyloid-like fibrils (*arrows*). **a**, **b**, **e**, **f** ×120,000; **c**, **d** ×60,000

The s-IBM vacuolated muscle-fiber cytoplasm, and often cytoplasm of non-vacuolated muscle fibers, also contain Aβ-immuno-positive: (a) collections of 6–10 nm filaments; (b) fine flocculomembranous material; and (c) amorphous material (Fig. 2e, f) [2, 5]. Only Aβ, and neither N- nor C-terminal epitopes of amyloid-β precursor protein (AβPP), is present on 6–10 nm amyloid-like filaments [2]. Myelin-like whorls and other lysosomal debris are present in the vacuolated fibers. Ultrastructurally *abnormal mitochondria containing paracrystalline inclusions* are occasionally present but not specific for s-IBM muscle fibers.

Our current concepts of s-IBM pathogenesis

Characteristic features of s-IBM muscle-fiber degeneration

We consider s-IBM essentially a degenerative muscle disease: (a) occurring within an aged muscle cellular environment, (b) associated with intracellular accumulation and aggregation of a number of proteins, (c) in conjunction with oxidative and endoplasmic-reticulum (ER) stresses, and (d) involving abnormal signal transduction and transcription. Those together lead to the IBM-specific muscle-fiber degenerative process.

In addition to muscle-fiber vacuolization and atrophy, degenerating s-IBM muscle fibers are characterized by accumulation of intra-muscle-fiber multiprotein aggregates ("inclusion-bodies"). In a given biopsy cross-section, the aggregates are present mainly in vacuole-free regions of vacuolated muscle-fiber cytoplasm and in cytoplasm of "non-vacuolated" fibers (because muscle fibers are individual cells often centimeters long, they might be vacuolated at another level). The vacuoles themselves usually do not contain the IBM-characteristic inclusions [12]. The s-IBM vacuoles are considered autophagic, since they often contain (a) lysosomal membranous debris, which is considered an end result of muscle-fiber destruction, and (b) increased immunoreactivity of some of the lysosomal enzymes [57]. The two major types of aggregates/inclusions in s-IBM

muscle fibers are: (a) the rounded, plaque-like aggregates prominently containing Aβ immunoreactivity, and (b) various-sized delicate, squiggly, linear aggregates containing p-tau, which are PHF by EM [12, 13]. Both types of aggregates contain proteins that are congophilic, indicating β-pleated-sheet configuration of amyloid [12]. And both also contain other proteins having a propensity to misfold, including α-synuclein and presenilin1 [4, 8, 12, also below]. Other characteristic features of the s-IBM muscle-fiber phenotype are evidence of proteasome inhibition, ER stress, and the unfolded-protein-response (UPR) [41, 72, 103, and below]. Accordingly, we consider that s-IBM is a conformational disease of muscle in which unfolding and misfolding of proteins within muscle fibers play a major role in cytotoxicity and the formation of multiprotein aggregates [11, 12]. We recently demonstrated activation of NF-κB [73], which also provides important insight into the s-IBM pathogenesis (below). We postulate that the *aging cellular milieu* in the s-IBM muscle fiber is important in promoting development of the characteristic progressive vacuolar degeneration and over-expression/accumulation of several potentially toxic proteins that may underlie disease progression (details below). Despite a concurrent over-expression of both putatively protective and damaging proteins and mechanisms [13], the defensive and reparative mechanisms are evidently insufficient because the muscle fibers continue to deteriorate and eventually die. Also contributing to progression might be that—compared to other myopathies including polymyositis and dermatomyositis—in s-IBM there is a definite paucity of regenerating muscle fibers ([67], and Askanas and Engel, personal observations).

Possible relationship between muscle-fiber degeneration and lymphocytic inflammation in s-IBM muscle biopsies

Also characteristic component of s-IBM muscle biopsies is a lymphocytic inflammation, mainly composed of cytotoxic CD8 cells [reviewed in 29]. Which component, degenerative or inflammatory, precedes the s-IBM pathogenesis [13, 29, 69] is a debated but unresolved issue. An earlier study reported that in cultured muscle cells, Aβ induces expression if IL-6 [17], supporting a primary, or possibly aggravating, role of Aβ in inducing an immune response. Conversely, a recent study reported that cytokine Il-1β induces AβPP in cultured muscle cells [82], supporting a primary, or perhaps an aggravating, role of inflammation in s-IBM [31]. Another argument given for primacy of the inflammatory component is that s-IBM muscle fibers abundantly express MHC-1, and muscle fibers expressing MHC-1 are invaded by clonally expanded cytotoxic CD8$^+$ lymphocytes [29, 30], as also occurs in polymyositis [29, 30]. However, MHC-1 expression is also present on regenerating muscle fibers in various other myopathies ([30] and Paciello and Askanas, unpublished observations, 2005). Moreover, MHC-1 is strongly expressed on non-regenerating muscle fibers in (a) genetic dysferlin deficiency, where it is associated with CD4 rather than CD8 T-lymphocyte response [21], and (b) in limb-girdle muscular dystrophy 2-I, where it is accompanied by inflammation [32]. In those two examples, presumably the genetic protein abnormally somehow leads to the MHC-1 expression. While MHC-1 is critical for a muscle fiber to become antigen-presenting, the mechanisms causing MHC-1 expression in s-IBM fibers are not known. There are several possible mechanisms. One is provocation by the acquired dysconfiguration of intramuscle fiber proteins (see below). Or possibly, MHC-1 expression in s-IBM results from the demonstrated ER stress ([72, 103], and see below), which additionally might be induced by AβPP that associates with ER chaperones GRP78 and GRP94 [103]. It has been shown that binding of ER chaperones to some peptides leads to expression of MHC-1, cellular antigen display, and activation of MHC-restricted T-cells [18, 89, 96]. ER stress recently has been shown to induce inflammatory and autoimmune responses [95, 119]. Very recently, we demonstrated activation of NF-κB in s-IBM muscle fibers [73] since NF-κB is a known proinflammatory factor [61], such NF-κB activation might contribute to s-IBM inflammation.

Relevant to treating patients, the main consideration in analyzing the s-IBM pathogenesis should focus less on whether inflammatory features are primary or secondary, and more on which of the pathologic abnormalities lead to muscle-fiber degeneration and weakness. It is now well-accepted that despite accumulation and activation of T-cells, s-IBM patients as a group respond poorly to anti-dysimmune treatment, in contrast to polymyositis patients, who have virtually identical immunopathologic abnormalities in their muscle biopsies [13, 29, 30, 35, 69]. This suggests that non-inflammatory factors are clinically more important.

We propose that the degenerative component within s-IBM muscle fibers (see details below) is pathogenically more important and is responsible for lack of response to various immune-modulating treatments. Accordingly, therapeutic considerations should focus on reducing detrimental degenerative components (suggestions below).

Possible detrimental role of various proteins abnormally accumulated within s-IBM muscle fibers

Intracellular toxicity of AβPP/Aβ

We have proposed for several years that increased *intracellular* expression of AβPP and of its proteolytic fragment Aβ play key upstream, toxic roles in the s-IBM pathogenesis [10, 12, 13]. Several experimental studies, including the

cultured human muscle IBM-model, and transgenic mouse models, provide strong evidence for an intracellular toxicity of AβPP/Aβ in s-IBM (recently reviewed in detail in [13]). Increased AβPP mRNA and abnormal accumulation of both AβPP and Aβ are identified early in s-IBM abnormal muscle fibers [10, 12]. In addition, there are abnormalities of the AβPP processing machinery. BACE1 and BACE2, which are glycosylated transmembrane β-*secretases* that cleave AβPP at the N-terminal of Aβ [49, 62, 86], are increased in s-IBM muscle fibers, where they are accumulated in the form of inclusions co-localizing with Aβ [104, 105]. BACE 1 also co-immunoprecipitates with AβPP in s-IBM muscle [113], suggesting that it may participate in AβPP processing and abnormal generation of the Aβ there. *Nicastrin* and *presenilins*, which are components of the *γ-secretase system* that cleaves AβPP at the C-terminal of Aβ, generating either Aβ40 or Aβ42 (reviewed in [87, 107]), are also strongly overexpressed in s-IBM muscle fibers, where they (a) colocalize with each other and with AβPP [85, 106], and (b) are physically associated with AβPP in both s-IBM and in experimentally AβPP-overexpressing muscle fibers (Vattemi and Askanas, unpublished observation, 2003). Accordingly, both β- and γ-secretases appear to participate in Aβ production within s-IBM muscle fibers. This probably would not be a problem if the Aβ would be properly disposed of (see below). AβPP/Aβ overexpressed in regenerating muscle fibers in various other muscle diseases does not seem to cause difficulty and is not associated with the s-IBM type of muscle-fiber degeneration [80, 105].Our most recent studies showed that in s-IBM muscle fibers AβPP is phosphorylated [93], and according to others, phosphorylation of AβPP increases its toxicity and assembly into Aβ toxic oligomers [23, 59].

Additionally, in s-IBM muscle fibers, there is preferential accumulation of the Aβ42 fragment [101], which is known to be more hydrophobic and more prone to self-association and oligomerization, and as such is much more cytotoxic than Aβ40 [39, 43, 44, 109]. Aβ cytotoxicity is considerably enhanced by its oligomerization. [39, 43, 44, 109]. In s-IBM muscle fibers, all congophilic (i.e., fibrillar, amyloidic) Aβ inclusions contain Aβ42 (Fig. 3, and Vattemi and Askanas, unpublished), while only some contain both Aβ40 and Aβ42 [101]. There are several other factors acting in s-IBM muscle fibers that might contribute to Aβ production, deposition and oligomerization. These include increased expression and accumulation of: (1) cystatin C (CC), an endogenous cysteine protease inhibitor, which was previously proposed to participate in Aβ deposition within the amyloid plaques AD brain [102]; (2) transglutaminases 1a and 2, which contribute to Aβ aggregation and insolubility by cross-linking Aβ molecules [26], and (3) free cholesterol [51], which increases Aβ production and amyloidogenesis (referenced in [51]). Our recent study [112] demonstrated that in s-IBM biopsied muscle and in AβPP-overexpressing cultured human muscle fibers, αB-crystallin (αBC), which specifically recognizes and stabilizes proteins that have a propensity to aggregate and precipitate [33, 88, 117], physically associated with AβPP and Aβ oligomers [112]. Therefore, binding of αBC to Aβ oligomers conceivably might retard and diminish their fibrillization and aggregation into visible non-toxic aggregates, thereby adversely prolonging their existence as toxic oligomers, increasing their cytotoxicity [112] (alternatively, that binding of αBC conceivably could detoxify Aβ).

Myostatin

Myostatin (MSTN) is a secreted protein, considered to be a negative regulator of muscle growth during development and of muscle mass during adulthood (reviewed in [52]). In biopsied s-IBM muscle fibers, MSTN precursor protein (MSTN-PP) and MSTN dimer were significantly increased; and MSTN-PP was physically associated with AβPP, and co-localized with Aβ by light- and electron-microscopic immunocytochemistry [111]. Moreover, AβPP-overexpression into cultured normal human muscle fibers increased MSTN-PP expression, and subsequent experimental inhibition of proteasome caused co-accumulation of both MSTN-PP/MSTN and AβPP/Aβ within aggresomes, and their physical association was evident by immunoprecipitation [114]. We proposed that AβPP binding to MSTN-PP causes its posttranslational modification that lessens its degradation and traffic, resulting in MSTN-PP accumulation.

Of particular interest are our recent studies demonstrating that in cultured human muscle fibers MSTN-PP mRNA and MSTN protein are significantly increased by NF-κB activation caused by experimentally induced ER stress [73]. The same mechanisms might contribute to the increase of MSTN in s-IBM muscle fibers, because: (a) ER stress is an important component of the s-IBM pathogenesis, and (b) the activity of NF-κB is increased in s-IBM muscle fibers [73]. Figure 4 illustrates our proposed mechanisms leading to MSTN increase and accumulation in s-IBM muscle fibers.

α-Synuclein (α-syn)

Abnormal expression of α-syn occurring spontaneously in brains of various neurodegenerative disorders has been associated with, and possibly causative of, oxidative stress, impaired proteasome function, and mitochondrial abnormalities [20, 27, 46, 63, 98]. We have shown (a) that α-syn is accumulated in s-IBM muscle fibers [4], and (b) that its 22 kDa O-glycosylated form is more expressed than its native 16 kDa form [76]. The 22 kDa form, but not the native 16 kDa form, was shown by others to be a target of ubiquitination by parkin [85]. The preferential increase of

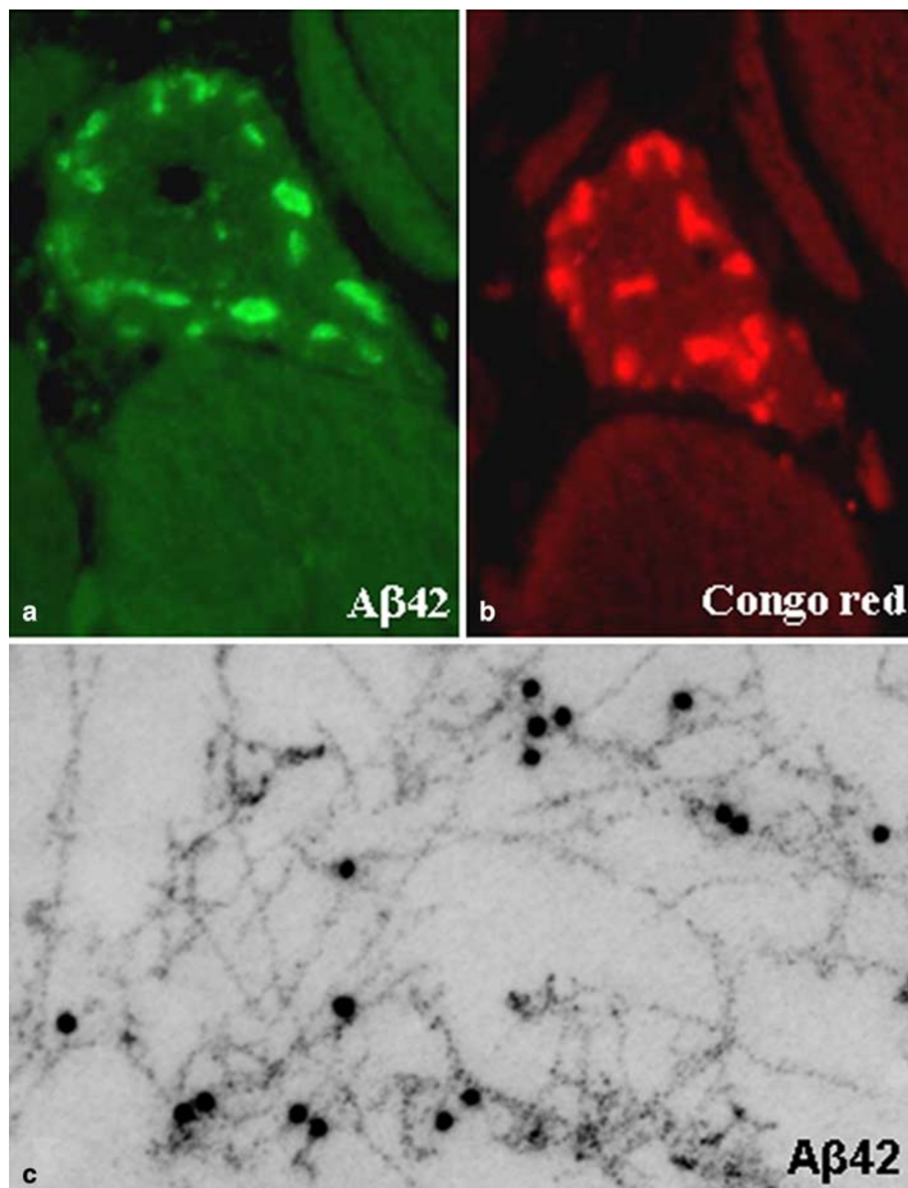

Fig. 3 Amyloid-β 42 (Aβ42) and Congo-red positivities in s-IBM muscle fibers. **a** Imunofluorescence and **c** gold-immuno-electronmicroscopy of Aβ42—stained with an antibody specifically recognizing Aβ42 (ref.97 here)—illustrate that Aβ42 aggregates in **a** correspond to 6–10 nm amyloid-like filaments in **c**. **b** Cong-red staining of a transverse parallel, but not closely adjacent, section of the same fiber as in **a**, demonstrating several amyloid inclusions. **a**, **b** ×2,100; **c** ×210,000

the 22 kDa O-glycosylated form of α-syn in s-IBM muscle fibers might be due to the proteasome inhibition we previously demonstrated in them [41, and below].

Because oxidative- and nitric-oxide-induced stress, and mitochondrial abnormalities, are also aspects of the s-IBM muscle-fiber pathology (reviewed in [12, 13]), a putative toxicity of α-syn, in addition to the cytotoxicity of Aβ and MSTN, may contribute to the muscle-fiber degeneration.

Parkin

This is an E3-ubiquitin ligase that ubiquitinates α-syn [81]. Parkin is increased in s-IBM muscle fibers, where it accumulates in the form of aggregates or aggresomes [76]. In brains of sporadic PD patients, parkin and α-syn accumulate in Lewy bodies, which are considered aggresomes [81]. Parkin, in addition to ubiquitinating several proteins, also protects cells against toxicity induced by α-syn, ER and other stresses, perhaps by helping to aggregate toxic α-syn oligomers and promote their degradation [50, 97]. Accordingly, we propose that increase of parkin in s-IBM muscle fibers is their attempt to protect themselves against toxicity induced by α-syn, ER and other stresses existing within themselves. However, the 2.7-fold increase of parkin in s-IBM muscle fibers might not be sufficient to overcome a sixfold increase of α-syn [76], or to protect against other continuing stresses. Accordingly, relative insufficiency of parkin could worsen the course of s-IBM. If so, manipulations toward increasing parkin might clinically benefit s-IBM muscle.

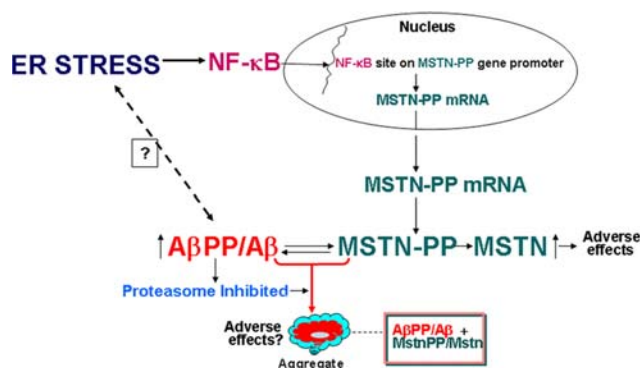

Fig. 4 Proposed pathologic regulation of myostatin-precursor protein (*MSTN-PP*)/myostatin (*MSTN*) in s-IBM muscle fibers. Endoplasmic-reticulum (*ER*) stress induces MSNT-PP transcription through activation of NF-κB. Increased MSTN then leads to muscle fiber atrophy. Furthermore, increased AβPP/Aβ, which also causes proteasome inhibition, binds to MSTN-PP/MSTN and both accumulate in the form of probably insoluble aggregates

Other important intracellular abnormalities in s-IBM muscle fibers

Decreased deacetylase activity of SIRT1

SIRT1 belongs to the mammalian sirtuin family of NAD^+-dependent histone deacetylases (HDACs) [45, 65, 100, 115]. Targets known to be deacetylated by SIRT1 include histone 4 (H4), NF-κB, and p53 [45, 65, 100, 115]. Through its deacetylase activity, SIRT1 is considered to control cellular metabolic homeostasis, and to play an important role in the regulation of gene expression, cell proliferation, differentiation, survival and senescence [45, 65, 100, 115].

SIRT1 activation has been considered to play a crucial role in the calorie-restriction (CR)-induced longevity in several species [65].

In addition, SIRT1 activation has been proposed to play a role in neuroprotection. For example, in an AD mouse model, increase of neuronal SIRT1 and its activation were reported to underlie the calorie-restriction prevention of Aβ-related AD-like neuropathology [78]. In various cell lines, increase of SIRT1 or its activation was reported to protect against Aβ toxicity by either decreasing the amount of Aβ by activating α-secretase [78], or by inhibiting NF-κB activation and its subsequent disturbance of signaling [24].

Our most recent studies have shown that, as compared to age-matched controls, in homogenates of s-IBM muscle fibers SIRT1 activity and deacetylation of SIRT1 targets NF-κB, H4, and p53 were significantly decreased despite increased SIRT1 protein expression [71]. Within isolated s-IBM muscle nuclei, there was decreased SIRT1 deacetylation activity accompanied by decreased SIRT1 protein expression in them [71]. Since increased acetylation (or decreased deacetylation) of NF-κB leads to its increased activity [116], decreased SIRT1 deacetylase activity might be directly responsible for the presumably detrimental NF-κB activation in s-IBM muscle fibers.

Our study provides, to our knowledge, the first demonstration of decreased SIRT1 deacetylase activity in any human muscle disease, viz., s-IBM, which is associated with aging. In well-differentiated cultured human muscle fibers, experimentally induced ER stress decreased SIRT1 activity and consequently increased NFκB acetylation (activation) [71]. Accordingly, in s-IBM muscle fibers, inadequate activity of SIRT1 may be detrimental by increasing NF-κB activation, and thereby contributing to the abnormal accumulation of Aβ and increased MSTN. Our proposed important consequences of decreased SIRT1 activity in s-IBM muscle fibers are illustrated in Fig. 5.

If correct, improving SIRT1 action by treatment with known SIRT1 activators might benefit s-IBM patients (see below).

Proteasome inhibition and aggresomes

The *26S proteasome*, an about 700 kDa multi-subunit protease complex present in the cytoplasm and nuclei of eukaryotic cells, has a major role in degrading normal and abnormal proteins through a ubiquitin-mediated process [108]. We have reported significant inhibition of the 26S proteasome function in (a) s-IBM muscle fibers, and (b) cultured human muscle fibers experimentally overexpressing Aβ/AβPP [41]. *Aggresomes*, which form when the

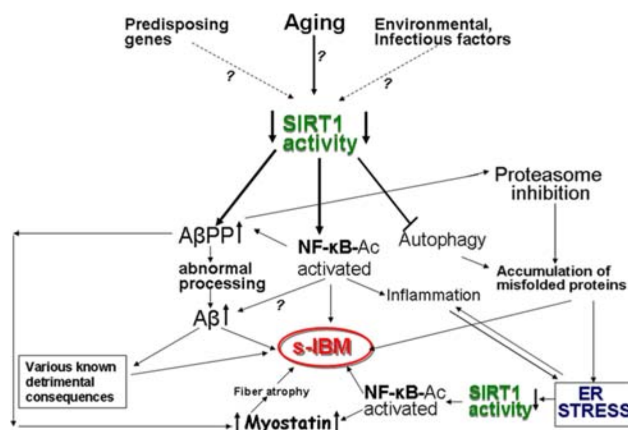

Fig. 5 Proposed adverse effects of decreased SIRT1 deacetylase activity in s-IBM muscle fibers. Decreased deacetylase activity of SIRT1 activates NF-κB by increasing its acetylation (NF-κB-Ac). This leads to increased myostatin, and other detrimental consequences. Decreased SIRT1 activity also increases AβPP and Aβ, resulting in their known detrimental effects in s-IBM muscle fibers, as detailed in the text. Decreased SIRT1 activity might also inhibit autophagy, contributing to the accumulation of multiprotein aggregates

proteasome is inhibited (referenced in [41]), are also part of the s-IBM muscle-fiber phenotype [41]; they were induced in cultured human muscle fibers by overexpressing AβPP ± proteasome inhibition [41].

In addition to increased Aβ/AβPP, other factors such as an aging muscle-fiber environment, protein overcrowding, oxidative stress, and accumulated p-tau, α-synuclein, and UBB^{+1} (referenced in [41] and below) might contribute to proteasome inhibition in s-IBM muscle fibers, resulting in accumulation of aggregated misfolded proteins into aggresomes. Furthermore, the unfolded/misfolded proteins might, in the putatively susceptible s-IBM patients, elicit expression and presentation of MHC-1 by the muscle fiber, and consequently induce a secondary CD8 T-cell response (see above).

Molecular misreading and accumulation of mutated ubiquitin (UBB^{+1})

"Molecular misreading" involves acquired, non-DNA-encoded dinucleotide deletions occurring within mRNAs, resulting in production of potentially toxic mutant proteins (recently reviewed in [99]). The aberrant transcripts are formed as a result of dinucleotide loss (ΔGA, ΔGU) during or after transcription, and they can be translated from the deletion onward into the +1 reading-frame to produce abnormal proteins, e.g., mutant ubiquitin, termed UBB^{+1}. The UBB^{+1} protein was shown to be accumulated in the dystrophic neurites as a component of neuritic plaques, and in neurofibrillary tangles of AD brain [99], as well as in brains of other neurodegenerative disorders in which inhibition of the proteasome has been proposed to play a pathogenic role [99]. The UBB^{+1} itself can become ubiquitinated, and then that form can inhibit the proteasome [99].

In s-IBM muscle fibers, UBB^{+1} was shown as being accumulated in the form of aggregates, providing the first demonstration that molecular misreading can occur in diseased human muscle [42]. We suggested that the aging cellular environment of s-IBM muscle fibers, combined with factors such as oxidative stress and perhaps other detrimental molecular events, leads to abnormal production and accumulation of UBB^{+1} [42]. Moreover, a high level of UBB^{+1} inhibits the 26S proteasome [99], and this mechanism might contribute to proteasome inhibition in s-IBM muscle fibers.

Endoplasmic-reticulum stress and the UPR

The ER is an intracellular compartment having a critical role in the processing, folding and exporting of newly synthesized proteins into the secretory pathway (reviewed in [95, 118, 119]). In the ER, molecular chaperones are required to assure proper folding of unfolded or misfolded proteins [95, 118, 119]. Unfolded proteins accumulating in the ER cause ER stress [95, 118, 119]. This elicits the UPR, a functional mechanism by which a cell attempts to protect itself against ERS [95, 118, 119]. In s-IBM muscle fibers, we have previously reported evidence of ER stress and the UPR [72, 103]. Recently, we demonstrated that in cultured normal human muscle fibers experimentally produced ER stress (a) induces MSTN through an NF-κB-related mechanism, and (b) decreases SIRT1 deacetylase activity (see above and [71, 73]). Accordingly, ER stress may importantly contribute to the s-IBM pathogenesis.

Mitochondrial abnormalities

These include: (a) ragged-red fibers [38], (b) cytochrome-*c*-oxidase (COX) negative muscle fibers, and (c) multiple mitochondrial DNA deletions (reviewed in [68, 74, 75]). These are more common in s-IBM muscle than expected for the patient's age [75, 79]. Our newest studies confirmed that COX-negative muscle fibers are significantly increased in s-IBM muscle biopsies and, although the COX-negative fibers are 90% Type-II, there is more involvement of Type-I fibers than in controls [91]. Our morphologically determined total percent of "COX-negative fibers" on transverse sections probably greatly underestimates their actual number because on longitudinal view the regions of COX-negativity are segmentally multifocal along the fibers ([75]; our unpublished observation). Although we previously showed in our IBM-model that excessive AβPP and Aβ contribute to the mitochondrial abnormalities [15] (a concept now supported by studies in other systems, especially as putatively related to AD and Parkinson's brain ([1, 46, 47] and referenced in [93]), our recent studies showed that COX-negative fibers do not preferentially contain aggregated Aβ and p-tau [91] and, as also recently reported [22], they do not correlate with foci of mononuclear inflammatory cells [91]. Accordingly, other yet unknown mechanisms seem to be causing the prominent COX-negativity in s-IBM muscle fibers. Possibilities include: toxic unaggregated oligomers of Aβ, α-syn, or other proteins; and factors resulting from oxidative or ER stresses. Discovery of their cause could facilitate developing treatment strategies. The mitochondrial abnormalities presumably contribute to the muscle-fiber malfunction and degeneration.

In the seemingly otherwise-intact muscle fibers, regions of COX-negativity cannot make ATP via oxidative-phosphorylation; those presumably weakened regions must be surviving on ATP diffusing from adjacent COX-positive regions or produced by anaerobic glycolysis.

DJ-1

The Parkinson-disease-related DJ-1 is a ubiquitously expressed protein of the ThiJ/PfpI/DJ1 superfamily (reviewed in [19, 25, 90] and referenced in [94]). We

recently reported that DJ-1 is increased in s-IBM muscle fibers, where it is (a) highly oxidized and (b) abnormally accumulated in muscle-fiber mitochondria [94]. Mutations in the DJ-1 gene that prevent expression of DJ-1 protein are a cause of early-onset autosomal-recessive PD [19, 25, 90]. In sporadic AD and PD brains, DJ-1 was reported to be increased and highly oxidized [25]. Although its precise functions are not yet known, DJ-1 has been proposed to act as an antioxidant ([90] and referenced in [94]) and be an important mitochondrial protective agent (referenced in [94]). Increased oxidation of DJ-1 itself was proposed to decrease its anti-oxidant activity ([90] and referenced in [94]). We suggest that in s-IBM muscle fibers the increased DJ-1 may be attempting to mitigate mitochondrial and oxidative damage, but its being excessively oxidized may render it ineffective [94].

Possible treatment avenues for s-IBM

Based on our studies, we propose that the most important general approach to developing treatment for s-IBM patients or to prevent progression of the disease is to stop deterioration and atrophy of the muscle fibers. The treatment approaches might be multifactorial, aiming toward various detrimental factors described above. Some approaches, based on our experimental IBM-culture models, appear promising. For example, our most recent studies demonstrated the following.

Resveratrol

Treatment with resveratrol of ER stress-induced cultured human muscle fibers (ER stress + IBM-culture-model) significantly decreased in them myostatin mRNA and protein, and was associated NF-κB de-acetylation (de-activation) [70]. Previously, resveratrol was shown to decrease Aβ and diminish AD neuropathology in AD mouse models [reviewed in 28]. Resveratrol (trans-3,4′,5-trihydroxystilbene), is an antioxidant polyphenol and a potent activator of SIRT1 (reviewed in [27]). Accordingly, resveratrol, and/or other small molecules that activate SIRT1, activity of which is decreased in s-IBM muscle [71], might be beneficial in treating s-IBM patients. Recently, SIRT1 activity has been reported to increase autophagy [60]. Although the exact role of autophagy in s-IBM awaits further studies, resveratrol possibly could also benefit s-IBM patients through induction of autophagy.

Lithium

Lithium has previously been shown to diminish tau and Aβ pathologies in various experimental models of AD (reviewed in [34]), but its clinical efficacy in treating AD patients is not established. In a transgenic mouse model whose skeletal muscle bears some aspects of IBM muscle fibers, lithium was reported to decrease tau phosphorylation through decreasing activity of GSK-3β [54].

Recently, we have shown that treatment of AβPP-overexpressing cultured human muscle fibers (AβPP + culture-IBM-model) with lithium significantly decreased total AβPP, phosphorylated AβPP, and Aβ oligomers [92]. In addition, lithium significantly increased the inactive form of GSK-3β and increased expression of an autophagosome marker LC3-II [92]. Accordingly, treating of s-IBM patients with lithium possibly could be beneficial.

Other possible treatments

Other approaches may involve the following. (1) Stopping hyper-phosphorylation of tau, which leads to its self-aggregation into PHFs, or blocking the aggregation process itself (and blocking any hypothetical binding of hyper-phosphorylated tau to normal cellular components). (2) Diminishing adverse effects of intra-muscle fiber cholesterol. However, the use of statins is of uncertain benefit and potentially myotoxic. (3) Reducing oxidative stress with various antioxidants. (4) Protecting mitochondria, especially ones not yet affected, perhaps with very high dose Coenzyne Q10 and L-carnitine (neither of proven efficacy), and with better protective molecules as they are developed. Greater understanding of molecular mechanisms associated with human muscle-fiber aging could provide new avenues toward s-IBM therapy.

Intriguing similarities of the s-IBM muscle fiber phenotype to those of brains of AD and PD

Similarities to the AD brain include accumulation of Aβ, phosphorylated tau (p-tau) and more than 15 other Alzheimer-characteristic proteins [8, 10, 12, 13]. For years it was considered that the *extra*cellular Aβ is exerting the main toxic, detrimental role in AD brain [83, 84]. However, more than a decade ago we proposed that our demonstrated *intra*cellular increase and toxicity of AβPP and of its proteolytic fragment Aβ play the key cytotoxic role in the s-IBM pathogenic cascade [14, 15]; furthermore, we postulated that the same *intra*cellular toxicity might be occurring in the AD pathogenesis [9]. This proposal regarding AD now seems to be gaining momentum, because more and more AD researchers discuss the possible importance of *intra*cellular Aβ accumulation and toxicity in the AD pathogenesis [reviewed in 43, 58].

Interestingly, there are also phenotypic similarities of s-IBM muscle fibers to the PD brain, such as accumulation

of α-syn, parkin and abnormalities of DJ-1, the latter also being present in AD brain [25]. These similarities suggest that the degenerative muscle and the brain diseases may share certain pathogenic steps and that knowledge of one disease might help elucidate the causes and treatments of the others. IBM, AD, and PD, including sporadic and hereditary forms of each, are all *multifactorial* and *polygenetic*. The respective cascade of events in each leading to their similar pathologic aspects is not well understood—cellular aging, protein misfolding, aggregation, proteasome inhibition, and mitochondrial abnormalities, as well as oxidative and ER stress have been proposed to be contributing in s-IBM, AD and PD [above, and reviewed in 1, 40, 48, 53, 56, 64, 74, 110]. Yet each disease category remains tissue- and region-specific, involving postmitotic-muscle fibers or postmitotic-neurons, thereby indicating that the mechanism of *cellular-targeting* is different in IBM, AD and PD. The tissue affected, muscle versus brain, may be influenced by: (1) etiologic agent (? a virus), (2) previous exposure to an environmental factor(s), (3) subtle differences of deficiency or toxicity factors, and (4) the patient's genetic background (the cellular microclimate). Easier availability of s-IBM patients' muscle biopsies, as compared to brain tissue, and the possibility of producing IBM experimental models by utilizing cultured human muscle fibers (which are the cells actually affected in the s-IBM disease process), might provide better understanding of some pathogenic aspects, not only related to s-IBM, but also to AD and PD, and facilitate development of treatments.

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