Short Communication

Endoplasmic Reticulum Stress and Unfolded Protein Response in Inclusion Body Myositis Muscle

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Proteins in the endoplasmic reticulum (ER) require an efficient system of molecular chaperones whose role is to assure their proper folding and to prevent accumulation of unfolded proteins. The response of cells to accumulation of unfolded proteins in the ER is termed “unfolded protein response” (UPR). UPR is a functional mechanism by which cells attempt to protect themselves against ER stress, resulting from the accumulation of the unfolded/misfolded proteins. Because intracellular inclusions, containing either amyloid- β ($A\beta$) or phosphorylated tau, are the characteristic feature of sporadic inclusion body myositis (s-IBM) muscle biopsies, we studied expression and immunolocalization of five ER chaperones, calnexin, calreticulin, GRP94, BiP/GRP78, and ERp72, in s-IBM and control muscle biopsies. Physical interaction of the ER chaperones with amyloid- β precursor protein ($A\beta$ PP) was studied by a combined immunoprecipitation/immunoblotting technique in s-IBM and control muscle biopsies, and in $A\beta$ PP-overexpressing cultured human muscle fibers. In all s-IBM muscle biopsies, all five of the ER chaperones were immunodetected in the form of inclusions that co-localized with amyloid- β . By immunoblotting, expression of ER chaperones was greatly increased as compared to the controls. By immunoprecipitation/immunoblotting experiments, ER chaperones co-immunoprecipitated with $A\beta$ PP. Our studies provide evidence of the UPR in s-IBM muscle and demonstrate for the first time that the ER chaperones calnexin, calreticulin, GRP94, BiP/GRP78, and ERp72 physically associate with $A\beta$ PP in s-IBM muscle, suggesting their playing a role in $A\beta$ PP folding and processing. (*Am J Pathol* 2004, 164:1–7)

Sporadic inclusion body myositis (s-IBM), the most common degenerative muscle disease of persons age 50 years and older, is of unknown etiology and pathogenesis.^{1,2} The main light-microscopic features of s-IBM muscle biopsies include: vacuolated muscle fibers; intra-muscle fiber inclusions; and various degrees of mononuclear cell inflammation.^{1,2}

An intriguing feature of the s-IBM muscle-fiber phenotype is its similarity to the Alzheimer's disease brain, including accumulation of amyloid- β ($A\beta$), phosphorylated tau, and several other Alzheimer characteristic proteins.^{1,2} Two major types of intracellular inclusion bodies in s-IBM muscle contain either $A\beta$ or phosphorylated tau.^{1,2} By light-microscopy inclusion bodies containing $A\beta$ are rounded and plaque-like, whereas those containing phosphorylated tau are more squiggly.^{1,2}

Both types of inclusion are positive with Congo Red, crystal violet, and thioflavin S, indicating that they contain proteins in alternate conformation (unfolded or misfolded) that are assembled in the β -pleated sheet configuration of amyloid.^{1,2} Ultrastructurally, amyloid- β -immunoreactive inclusions appear as aggregates of 6- to 10-nm amyloid-like fibrils and amorphous material, whereas inclusions containing phosphorylated tau appear as 15- to 21-nm paired-helical filaments.^{1,2} The cytoplasmic inclusion bodies are present mainly in vacuole-free regions of the vacuolated muscle fibers and in nonvacuolated muscle fibers.

Both types of inclusions contain several other accumulated proteins, some of which are present in each.^{1,2} Some, such as α -synuclein and cellular prion protein, have, similarly to $A\beta$ and tau, a propensity to unfold, misfold, and form β -pleated sheet amyloid.^{3,4} It has been proposed that unfolding and misfolding of proteins play a role in the formation of the multiprotein aggregates (inclusions) within the IBM fibers.²

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The endoplasmic reticulum (ER) is an intracellular compartment having a critical role in the processing, folding, and exporting of newly synthesized proteins into the secretory pathway.⁵ Folding of proteins in the ER requires an efficient system of molecular chaperones whose role is to assure proper folding of misfolded proteins in ER.⁶ Unfolded proteins accumulating in the ER lead to endoplasmic reticulum stress (ERS). This elicits the unfolded protein response (UPR), a functional mechanism by which cells attempt to protect themselves against ERS.^{6,7} The UPR involves transcriptional induction of ER chaperone proteins whose function is both to increase folding capacity of the ER and prevent protein aggregation^{6,7}; translational attenuation to reduce protein overload and subsequent accumulation of unfolded proteins^{6,7}; and removal of misfolded proteins from the ER through retrograde transport coupled to their degradation by 26S proteasome.⁸

In this study we investigated whether the accumulated unfolded/misfolded proteins in s-IBM muscle induce ERS and the UPR by studying five ER chaperones, calnexin, calreticulin, BiP/GRP78, GRP94, and ERp72, using light- and electron-microscopic immunocytochemistry and immunoblotting. By a combined immunoprecipitation/immunoblotting technique, we studied the physical interaction of the ER chaperone proteins with A β PP both in s-IBM muscle and in A β PP-overexpressing cultured human muscle fibers, because our previous studies demonstrated that intramuscle fiber accumulation of A β PP/A β appears to be an important upstream step in the IBM pathogenic cascade.^{1,9,10}

Materials and Methods

Muscle Biopsies

Immunocytochemical studies were performed on 10- μ m-thick unfixed sections of fresh-frozen diagnostic muscle biopsies obtained (with informed consent) from 25 patients with these diagnoses: s-IBM ($n = 10$), dermatomyositis ($n = 3$), polymyositis ($n = 3$), morphologically non-specific myopathy ($n = 3$), and normal muscle ($n = 6$). Diagnoses were based on clinical and laboratory investigations, including our routinely performed 18-reaction diagnostic histochemistry of the biopsies.¹¹ All IBM biopsies showed muscle fibers with vacuoles on Engel trichrome staining¹² and 15- to 21-nm paired helical filaments (PHFs) by electron microscopy and by SMI-31 immunoreactivity.¹³ In addition, all s-IBM patients had, in 60 to 80% of their vacuolated muscle fibers, Congo Red positivity using fluorescence enhancement.¹⁴

Light-Microscopic Immunocytochemistry

Immunocytochemistry was performed on transverse sections of the freshly frozen muscle biopsies. Peroxidase-anti-peroxidase and immunofluorescence procedures were as described,^{15–18} using the following well-characterized antibodies: rabbit polyclonal (Stressgen, Victoria, Canada) and mouse monoclonal (BD Transduction Lab-

oratories, San Diego, CA) antibodies against calnexin, diluted 1:1000 and 1:500, respectively; rabbit polyclonal antibody against calreticulin (Affinity Bioreagent, Golden, CO), diluted 1:1000; mouse monoclonal antibody against BiP/GRP78 (BD Transduction Laboratories), diluted 1:20; rabbit polyclonal antibody against GRP94 (Stressgen), diluted 1:50; and mouse monoclonal antibody against ERp72 (BD Transduction Laboratories), diluted 1:20.

Double immunofluorescence used antibodies against calnexin, calreticulin, and GRP94 in combination with mouse monoclonal antibody 6E10 (Signet, Dedham, MA), diluted 1:100. 6E10 recognizes A β in both Alzheimer's disease brain¹⁹ and IBM muscle¹⁶ by light- and electron-microscopic studies, and on immunoblots it recognizes the A β region within the parent A β PP molecule as well as free A β 40 and A β 42. In addition, on serial sections immunoreactivities of BiP/GRP78 and ERp72 were compared to A β immunolocalized by 6E10 antibody.

To block nonspecific binding of antibody to Fc receptors, sections were preincubated with normal goat or rabbit serum diluted 1:10, as described.^{15–18} Controls for staining specificity were omission of the primary antibody, or its replacement with nonimmune sera or irrelevant antibody.

Immunoelectron Microscopy

This was done on 10- μ m unfixed frozen sections adhered to the bottom of 35-mm Petri dishes, as detailed.^{15–18} In brief, after incubation with a primary antibody against calnexin, calreticulin, BiP/GRP78, GRP94, or ERp72, followed by washing and incubation in the appropriate secondary antibody conjugated to 10-nm gold particles, the sections were fixed in a 2% paraformaldehyde-1.25% glutaraldehyde mixture, postfixed in 1% osmium tetroxide, and Epon-embedded *in situ* in the Petri dish. Muscle fibers immunopositive for calnexin, calreticulin, BiP/GRP78, GRP94, or ERp72 were light-microscopically identified on peroxidase-anti-peroxidase-stained adjacent sections. The embedded sections in the dish were viewed under phase-contrast microscopy, and the same previously identified muscle fibers were marked, drilled-out, and processed for electron microscopic studies, as described.^{15–18}

Immunoblotting

Western blot analysis was performed as described.^{17,18} Protein concentration was measured by the Bradford method. Twenty μ g of protein was loaded on 7.5% polyacrylamide gel, separated by electrophoresis, and then transferred to a nitrocellulose membrane. Nitrocellulose membranes were blocked in 5% (w/v) blocking reagent (Amersham, Piscataway, NJ) in phosphate-buffered saline plus 0.1% Tween 20, and were incubated overnight at 4°C with one of the following antibodies: calnexin, diluted 1:2000 (Stressgen) and 1:1000 (BD Transduction Laboratories); calreticulin, diluted 1:2000; BiP/GRP78, diluted 1:200; GRP94, diluted 1:500; and ERp72, diluted 1:200. After being washed, the membrane was incubated

with the secondary antibody conjugated to horseradish peroxidase. The blots were developed using an enhanced chemiluminescence system (Amersham). Protein loading was evaluated by the actin band visualized with a monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

Combined Immunoprecipitation/Immunoblot Procedure

To evaluate whether calnexin, calreticulin, BiP/GRP78, GRP94, and ERp72 physically associate with A β PP, a combined immunoprecipitation/immunoblot technique was performed, as we recently described.¹⁸ In brief, 100 μ g of total muscle protein from s-IBM and control biopsies was diluted in 1 ml of precipitation buffer (1% Triton X-100, 150 mmol/L NaCl, 10 mmol/L Tris, pH 7.4, 1 mmol/L EDTA, 1 mmol/L EGTA, pH 8.0, 0.2 mmol/L sodium orthovanadate, 0.2 mmol/L phenylmethyl sulfonyl fluoride, 0.5% Nonidet P-40). Five μ g of 6E10 antibody was then added. The reaction mixture was incubated overnight at 4°C on a orbital shaker. Ten μ l of 50% Protein A:Agarose (BD Transduction Laboratories) was added, and the reaction mixture was incubated for 2 hours at 4°C. That solution was centrifuged for 4 minutes ($16,000 \times g$ at 4°C) and the supernatant removed. The precipitated immunocomplex was washed twice with the precipitation buffer by centrifuging 4 minutes ($16,000 \times g$ at 4°C). The pellet was resuspended in 30 μ l of 2 $\times$ concentrated sample buffer, boiled for 5 minutes, and centrifuged for 5 minutes ($16,000 \times g$ at 4°C). Ten μ l of the supernatant was loaded onto sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and separated by electrophoresis. Gels were then transferred to a nitrocellulose membrane and blotted with calnexin, calreticulin, BiP/GRP78, GRP94, or ERp72 antibody, diluted as described in the immunoblotting section. Then an appropriate horseradish peroxidase-labeled secondary antibody and enhanced chemiluminescence Western blotting detection reagents (Amersham) were used. To confirm the specificity of those experiments the following controls were performed: 1) the antibody against A β PP, which is used for immunoprecipitation, was omitted. This resulted in a total nondetection of co-immunoprecipitated proteins (see Figure 3). 2) The primary antibodies used for immunoblotting of immunoprecipitates were omitted, and in these cases immunoblots were reacted only with the secondary antibodies directed against either a monoclonal mouse or a polyclonal rabbit antibody. This immunoblotting of the immunoprecipitates did not produce any specific bands (see Figure 3).

Cultured Human Muscle Fibers

Tissue cultures of normal human muscle were established, as we have described, from satellite cells of portions of diagnostic muscle biopsies from patients who, after all tests were performed, were considered free of muscle disease.²⁰ Cultures were maintained as recently described.¹⁸

The A β PP-gene was transferred into 2-week-old cultured muscle fibers using an adenovirus vector, as described.^{9,10} Four days after the A β PP gene transfer, A β PP-overexpressing and control cultures were processed for the combined immunoprecipitation/immunoblot studies as described above for biopsied s-IBM and control muscle.

Results

Light-Microscopic Immunocytochemistry

s-IBM

In all s-IBM muscle biopsies, 70 to 80% of the vacuolated muscle fibers contained, mainly in their nonvacuolated cytoplasm, numerous well-defined, various sized, plaque-like, dotted, or elongated inclusions strongly immunoreactive with the antibodies against calnexin, calreticulin, ERp72, BiP/GRP78, and GRP94 (Figure 1). In addition, in all biopsies, 10 to 15% of the nonvacuolated, otherwise normal-appearing (on a given cross-section) fibers contained similar inclusions. By double-label fluorescence immunocytochemistry, in all abnormal muscle fibers, inclusions immunoreactive for calnexin, calreticulin, and GRP94 co-localized with A β -immunoreactivity (Figure 1). On serial sections pairs stained for BiP/GRP78 and A β , or ERp72 and A β , A β immunoreactivity was present in the same muscle fibers as those having ER chaperone immunoreactivity (not shown).

Other Diseased and Normal Human Muscle

None of the control normal or diseased biopsies had muscle fibers containing s-IBM characteristic inclusions immunoreactive for calnexin, calreticulin, BiP/GRP78, GRP94, and ERp72. Specifically, regenerating and degenerating muscle fibers in the disease controls were not immunoreactive. When primary antibody was omitted or replaced with nonimmune serum or irrelevant antibody, the above-described immunoreactions were not evident.

Immunoelectron Microscopy

In s-IBM abnormal muscle fibers, calnexin, calreticulin, BiP/GRP78, GRP94, and ERp72 were prominently accumulated on 6- to 10-nm amyloid-like fibrils and on electron-dense amorphous material (Figure 2). In addition, each of those proteins was present on small patches on amorphous material and 6- to 10-nm filaments surrounding clusters of PHFs, but was not directly immunolocalized to the PHFs themselves (Figure 2).

Immunoblots

In normal and s-IBM muscle biopsies calnexin migrated as a 90-kd band, calreticulin as a 60-kd band, and BiP/GRP78 as a 78-kd band, as previously described.^{6,21} Their expression was much stronger in s-IBM than in normal control muscle biopsies (Figure 3). In normal and

Figure 1. Immunofluorescence of ER chaperones in s-IBM muscle. **A–E:** Single-label immunofluorescence illustrates strongly immunoreactive, various-sized inclusions of calnexin, calreticulin, ERp72, BiP/GRP78, and GRP94 in s-IBM vacuolated muscle fibers. Most of the inclusions are present in the vacuole-free cytoplasm. **F–K:** Double-label immunofluorescence illustrates that in s-IBM muscle fibers the inclusions immunoreactive for calnexin (**F**), calreticulin (**H**), and GRP 94 (**J**) were also immunoreactive for amyloid- β (**G**, **I**, **K**). Original magnifications, $\times 1100$.

s-IBM muscle biopsies, ERp72 migrated as 69- and 72-kd bands, and the intensity of the 72-kd band was much stronger in s-IBM biopsies than in normal muscle (Figure 3). A 94-kd band corresponding to GRP94 was present in s-IBM, but not in the normal muscle (Figure 3).

Immunoprecipitation

Immunoprecipitation of s-IBM and control muscle biopsies with the mouse monoclonal 6E10 antibody, which can recognize either A β or A β PP, followed by Western blotting and immunoprobings with antibodies individually against calnexin, calreticulin, BiP/GRP78, GRP94, and ERp72, revealed expected bands corresponding to calnexin, calreticulin, BiP/GRP78, GRP94, and ERp72 in

s-IBM muscle, while control muscle did not detectably express any of these bands (Figure 3). Control experiments were negative (Figure 3).

Cultured Human Muscle Fibers

Immunoblots of the 3-week-old control cultured control human muscle fibers had 90-, 60-, 78-, and 94-kd bands corresponding to calnexin, calreticulin, BiP/GRP78, and GRP94, respectively, and 72- and 69-kd bands of ERp72 (Figure 3). In the A β PP-overexpressing cultured human muscle fibers, immunoprecipitation with 6E10 antibody followed by Western blotting and immunoprobings with antibodies against calnexin, calreticulin, BiP/GRP78, GRP94, or ERp72, revealed the expected bands similarly

Figure 2. Gold immunoelectron microscopy in s-IBM muscle fibers using 10-nm gold particles. **A–C,** Calnexin; **D and E,** calreticulin; **F and G,** ERp72; **H–J,** GRP94. All illustrated ER chaperones localized mainly to 6- to 10-nm amyloid-like fibrils (**A**, **D**, **F**, **H**) and floccular and amorphous material (**B**, **D**, **I**, **G**). They also localized at the periphery of the bundles of PHFs, but not on the PHFs themselves (**C**, **E**, **J**). Original magnifications: $\times 42,500$ (**A**, **C**, **D**, **F**, **H**, **I**); $\times 33,400$ (**B**, **G**); $\times 53,400$ (**E**, **J**).

Figure 3. Immunoblots and immunoprecipitations of normal control and s-IBM muscle biopsies (**top**), and normal and A β PP-overexpressing cultured human muscle fibers (**bottom**). Muscle biopsies: immunoblots (IM) of muscle homogenates of normal control (C) and s-IBM muscle biopsies (**top row**) demonstrate in s-IBM a much stronger expression of calnexin (90 kD), calreticulin (60 kD), BiP/GRP78 (78 kD), ERp72 (doublet of 72 and 69 kD), and GRP94 (94 kD) as compared to control muscle biopsies. The actin band shows the protein loading in each specimen. Immunoprecipitation (IP) of control and s-IBM muscle biopsies. IP-1: Immunoprecipitation with 6E10 antibody followed by immunoprobings (IM) with antibodies against calnexin, calreticulin, BiP/GRP78, ERp72, and GRP94 reveals strong bands of the appropriate molecular weight corresponding to the given ER chaperones in s-IBM muscle, whereas controls do not express any bands. IP-2: Immunoprecipitation with omission of 6E10 antibody, followed by immunoprobings (IM) with antibodies against calnexin, calreticulin, BiP/GRP78, ERp72, and GRP94 does not reveal any bands. IP-3: Immunoprecipitation step as in IP-1, but immunoblots were reacted only with the secondary antibodies directed against either a monoclonal mouse or a polyclonal rabbit antibody. This immunoblotting of the immunoprecipitates does not produce any specific bands. Culture muscle fibers: immunoblots (IM) of two homogenates of 3-week-cultured human muscle fibers shows expression of calnexin, calreticulin, BiP/GRP78, ERp72, and GRP94. Immunoprecipitation (IP) of control (C) and A β PP-overexpressing (A β PP+) cultured human muscle fibers, IP with 6E10 antibodies followed by immunoprobings with antibodies against calnexin, calreticulin, BiP/GRP78, ERp72, and GRP94 shows, in the A β PP+ cultured muscle fibers, bands of the appropriate molecular weight corresponding to each ER chaperone, whereas controls do not express any bands. IP with antibodies against calreticulin, BiP/GRP78, and GRP94, followed by immunoprobings with 6E10 antibody shows in the A β PP+-cultured muscle fibers a 130-kD band corresponding to A β PP, while controls are negative.

to the s-IBM-biopsied muscle. Immunoprecipitation of control cultured human muscle fibers did not reveal any bands (Figure 3). In the reverse experiments, immunoprecipitation with the antibodies individually against calnexin, calreticulin, BiP/GRP78, and GRP94, followed by Western blotting and immunoprobings with anti-A β mouse monoclonal 6E10 antibody, revealed an ~130-kD band corresponding to the mature form of A β PP. Control cultured human muscle fibers did not have any bands corresponding to A β PP (Figure 3). Considered together, these studies suggest that in A β PP-overexpressing cultured human muscle fibers, similarly to the s-IBM-biopsied muscle, calnexin, calreticulin, BiP/GRP78, GRP94, and ERp72 co-immunoprecipitate with A β PP.

Discussion

UPR in s-IBM Muscle

Our immunoblot studies demonstrate that expression (meaning detectability) of five ER-chaperone proteins—calnexin, calreticulin, BiP/GRP78, GRP94, and ERp72—is increased in s-IBM muscle biopsies. This could be reflecting increased genetic expression or decreased turnover of the protein. Our immunocytochemical studies demonstrate that these ER chaperones are abnormally, multifocally accumulated in s-IBM muscle fibers, where they co-localize with A β by light microscopy. By gold immunoelectron microscopy, the ER chaperones immunolocalize to 6- to 10-nm amyloid-like fibrils and to floccular/amorphous material. In s-IBM muscle fibers, the 6- to 10-nm amyloid-like fibrils are known to contain immunoreactive A β but lack the C- and N-termini of A β PP; whereas, the floccular/amorphous material, contains both A β and the C- and N-termini of A β PP, and is proposed to represent insoluble but less-organized amyloid or preamyloid.¹⁵ Accordingly, in s-IBM muscle fibers, ER chaperones associate with structures containing A β and A β PP. Moreover, our immunoprecipitation results demonstrate, in s-IBM muscle and in the A β PP-overexpressing cultured human muscle fibers, that the five ER chaperones physically interact with A β PP—this suggests they possibly play a role in folding, or attempted folding, of A β PP/A β in s-IBM muscle.

The Presence of UPR in s-IBM Muscle Fibers Suggests that Unfolded Proteins Are Contributing to s-IBM Pathogenesis

The ER-resident chaperone proteins up-regulated as part of the UPR are in three main groups, each of which has been investigated in this study. One group includes peptide-binding molecular chaperones BiP/GRP78, GRP94, and GRP170.^{6,22} BiP/GRP78 and GRP94, the most well-characterized peptide-binding proteins, interact transiently with protein-folding intermediates to prevent aggregation of a protein by keeping it in a folding-competent state.^{6,22} Interaction between the chaperones and proteins ensures that only proteins that are properly assembled and folded leave the ER compartment.⁶ The second group is comprised of the disulfide isomerase family, including PDI, ERp72, ERp59, and ERp29.⁶ These

are involved in protein folding by functioning as oxidoreductases in the formation/isomerization of disulfide bonds.²³ In addition, PDI, ERp72, ERp59, and ERp29 also have peptide-binding activity.²³ The third group includes calreticulin and calnexin, two homologous lectins that bind transiently to newly synthesized, not yet folded glycoprotein intermediates.^{5,6} They bind to glycoproteins only when they have monoglucosylated N-glycan, and they promote folding, delay oligomerization, and prevent degradation of them.^{21,24}

Unfolding or misfolding of proteins can occur *in vivo* and *in vitro* under several circumstances, including macromolecular crowding, oxidative stress, exposure to toxins, and aging.^{25,26} In s-IBM muscle, there is increased transcription of several genes, eg, A β PP-751, cellular prion protein, enzymes involved in cellular defense against oxidative stress, and c-Jun.^{1,2} There is also abnormal accumulation of RNA, ectopic expression of RNA-polymerase II, and abnormalities of signal transduction.^{1,2} These phenomena might be contributing to abnormal accumulation and crowding of proteins, which could lead to their unfolding, abnormal glycosylation, and other consequences deleterious to muscle-cell proteins and their vital functions. Even though the s-IBM inclusions contain several aggregated proteins, A β PP/A β appears to play a key role in IBM pathogenesis. For example, in s-IBM muscle: A β PP/A β accumulation appears to precede other abnormalities and large intracellular inclusions composed of A β are congophilic, indicating its misfolded, β -pleated sheet configuration as amyloid.^{1,2} Moreover, experimentally induced intracellular overexpression of A β PP in cultured human muscle fibers leads to aspects of the IBM phenotype in them, including A β PP/A β -containing congophilic inclusions, abnormal mitochondria, and aspects of oxidative stress.^{1,9,10} Our present study does not preclude that in s-IBM muscle ER chaperones might be associated with other accumulated proteins, but, by our immunoprecipitation-immunoblotting, it demonstrates for the first time their physical association with A β PP, both in s-IBM muscle and in A β PP-overexpressing cultured muscle fibers. This suggests their participation in the folding and removal of the excessively synthesized A β PP, and/or of its proteolytic product A β . Although binding of calreticulin and BiP/GRP78 to A β PP has previously been reported in other systems,^{27–29} our studies are the first to demonstrate that calnexin, GRP94, and ERp72 physically associate with A β PP, which suggests an importance of ER chaperones in A β PP folding.

We propose that the ERS is part of the IBM pathogenic cascade, occurring in response to abnormally unfolded or misfolded proteins; and that the UPR, evidenced by increased expression of the five ER chaperones calnexin, calreticulin, BiP/GRP78, GRP94, and ERp72, is an attempt to facilitate proper folding of malformed proteins, and/or their disposal.

However, the presence of the amyloid-positive (ie, congophilic) inclusions, indicates that the UPR is not completely effective. This may be related, at least partially, to an inhibition in s-IBM muscle of the 26S proteasome, which participates in the disposal of malformed

proteins. Our recent studies demonstrated that mutated ubiquitin, termed UBB⁺, previously shown to inhibit 26S proteasome activity in other systems,³⁰ is abnormally accumulated in s-IBM muscle fibers.³¹ Future experimental studies should elucidate the possibility of a functional relationship between putative proteasome inhibition and the demonstrated ER stress and the UPR observed in s-IBM muscle.

Therapeutic Perspective

New, putatively pathogenic phenomena have been identified in s-IBM. They suggest that one would like to stop the intracellular increase of the unfolded/misfolded proteins by reducing their formation and/or increasing their disposal. How to do this is now the challenge.

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