REVIEW ARTICLE

Progress in Understanding the Pathogenesis of Oculopharyngeal Muscular Dystrophy

Xueping Fan, Guy A. Rouleau

ABSTRACT: Oculopharyngeal muscular dystrophy (OPMD) is an adult-onset disorder characterized by progressive eyelid drooping (ptosis), swallowing difficulties (dysphagia), and proximal limb weakness. The autosomal dominant form of this disease is caused by expansions of a $(GCG)_6$ repeat to $(GCG)_{8-13}$ in the PABPN1 gene. These mutations lead to the expansion of a polyalanine stretch from 10 to 12-17 alanines in the N-terminal domain of PABPN1. Mutated PABPN1 (mPABPN1) induces the formation of muscle intranuclear inclusions that are thought to be the hallmark of this disease. In this review, we discuss: 1) OPMD genetics and PABPN1 function studies; 2) diseases caused by polyalanine expansions and cellular polyalanine toxicity; 3) mPABPN1-induced intranuclear inclusion toxicity; 4) role of oligomerization of mPABPN1 in the formation and toxicity of OPMD intranuclear inclusions and; 5) recruitment of subcellular components to the OPMD inclusions. We present a potential molecular mechanism for OPMD pathogenesis that accounts for these observations.

RÉSUMÉ: Progrès dans la compréhension de la pathogenèse de la dystrophie musculaire oculopharyngée. La dystrophie musculaire oculopharyngée (DMOP) est une maladie de l'âge adulte caractérisée par une chute progressive de la paupière supérieure (ptose), des difficultés de déglutition (dysphagie) et une faiblesse musculaire proximale. La forme autosomique dominante de la maladie est causée par une expansion de répétitions GCG de 6 à 8-13 répétitions dans le gène PABPN1. Ces mutations provoquent l'expansion d'une séquence de polyalanine de 10 à 12-17 alanines dans le domaine n-terminal de PABPN1. Le gène PABPN1 muté (mPABPN1) entraîne la formation d'inclusions intranucléaires musculaires qui sont considérées comme les stigmates de cette maladie. Dans cette revue, nous discutons: 1) de la génétique de la DMOP et des études de fonction du gène PABPN1; 2) des maladies causées par une expansion polyalanine et de la toxicité cellulaire de la polyalanine; 3) de la toxicité des inclusions intranucléaires dues à mPABPN1; 4) du rôle de l'oligomérisation de mPABPN1 dans la formation et la toxicité des inclusions intranucléaires de la DMOP et 5) du recrutement de composantes subcellulaires aux inclusions de la DMOP. Nous présentons un mécanisme moléculaire de la pathogenèse de la DMOP qui expliquerait toutes ces observations.

Can. J. Neurol. Sci. 2003; 30: 8-14

Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant, adult-onset disease with a worldwide distribution that has been reported in at least 33 countries. Several clusters of OPMD have been found, the largest being in French-Canadian population where the estimated mutation-carrier prevalence is in the order of 1:1000.[1,2] The highest disease gene frequency is found in Bukhara Jews now settled in Israel, where the prevalence is close to 1:600. In the French general population, the suggested prevalence is 1:100,000, similar to that in much of the rest of Europe.[2,3] Oculopharyngeal muscular dystrophy usually presents in the fifth decade with progressive swallowing difficulty (dysphagia), eyelid drooping (ptosis), and proximal limb weakness.[3] Swallowing difficulties may become debilitating with pooling of secretions in the nasopharynx and episodes of aspiration. Disease progression varies widely from one individual to another. A few patients become wheelchair bound due to the combination of marked proximal limb-muscle weakness, dysphagia-related cachexia and aging.[2,3]

In 1980, Tome and Fardeau[4] first identified a unique accumulation of nuclear filaments in OPMD muscle fibers. Later reports further confirmed the presence of the intranuclear inclusions in muscle nuclei.[5-9] Under an electron microscope, OPMD inclusions are seen as filaments that have a tubular appearance with an outer diameter of 8.5 nm, an inner diameter of 3 nm, and a length of approximately 0.25 μm. These unique

From the Center for Research in Neuroscience, McGill University, and the McGill University Health Center, Montreal, Quebec, Canada

Received January 17, 2002. Accepted in final form August 12, 2002.

Reprint requests to: Guy A. Rouleau, Rm L7-224 , Montreal General Hospital, 1650 Cedar Avenue, Montreal, Quebec H3G 1A4, Canada

intranuclear filament inclusions in skeletal muscle fibers are the pathological hallmark of OPMD.[4]

Oculopharyngeal muscular dystrophy is usually inherited as an autosomal dominant trait with complete penetrance and without gender preference. The OPMD locus was mapped to chromosome 14q11.2-q13 by linkage analysis[1,10-12] and the mutated gene positionally cloned and identified as the poly(A) binding protein nuclear 1 (PABPN1, also known as PABP2). Oculopharyngeal muscular dystrophy is caused by the expansion of the short (GCG) trinucleotide repeat in the coding sequence of the PABPN1 gene. The normal PABPN1 gene has a $(GCG)_6$ repeat encoding a polyalanine stretch at the 5'end, while in OPMD patients this repeat is expanded to $(GCG)_{7\text{-}13}$. Expansions of $(GCG)_{8\text{-}13}$ cause autosomal dominant OPMD, whereas homozygosity for the $(GCG)_7$ allele leads to autosomal recessive OPMD. Due to the presence of a GCA GCA GCA GCG coding sequence adjacent to the $(GCG)_6$ repeat, the wild-type PABPN1 protein has a 10 alanine stretch, whereas the mutated PABPN1 (mPABPN1) has 11 to 17 alanines in the N-terminal domain.[13] Polyalanine stretch expansions from 10 to 12-17 alanines cause dominant OPMD.[13] Compound heterozygotes with a $(GCG)_7$ and a $(GCG)_{8\text{-}13}$ allele and homozygotes with $(GCG)_{8\text{-}13}$ have an earlier disease onset, suggesting a dosage effect.[13]

PABPN1 has 306 amino acids and comprises an alanine stretch and a proline-rich region in the N-terminus, a putative ribonucleoprotein (RNP)-type RNA binding domain in the central region, and an arginine-rich C-terminal domain. PABPN1 is an abundant nuclear protein, binds with high affinity to the poly(A) tail of mRNA, and is involved in mRNA polyadenylation.[14] Polyadenylation is a two-step reaction whereby endonucleolytic cleavage of the nascent mRNA transcript is followed by the addition of ~250 adenylate residues to the up-stream cleavage product.[15-17] Poly(A) tail synthesis is catalyzed by poly(A) polymerase through interaction with CPSF, the cleavage and polyadenylation specificity factor. However, this process is slow and inefficient, and the length of poly(A) tail is poorly controlled. Adding PABPN1 to this reaction will stimulate processive poly(A) addition and control the size of the tail to be ~250 nucleotides in length.[14,18,19] The RNA binding domain mediates its specific binding to the poly(A) tail of mRNA.[20] Poly(A) bound PABPN1 forms both linear filaments and discrete-sized, compact oligomeric particles *in vitro*.[21] Titration and gel retardation assays indicated that 12 adenylate residues are required for high affinity RNA binding and the packing density on the poly(A) tail is approximately 15 adenylate residues per PABPN1 molecule.[20] However, PABPN1 tends to form oligomers even in the absence of mRNA.[22]

Polyalanine stretch expansions and diseases

In addition to OPMD, at least five other diseases are associated with polyalanine stretch expansions in disease gene products. Synpolydactyly is a dominantly inherited congenital limb malformation that typically consists of 3/4 syndactyly in the hands and 4/5 syndactyly in the feet with digit duplication in the syndactylous web.[23] Synpolydactyly results from a polyalanine stretch expansion in the protein HOXD13 and is the first human malformation syndrome shown to be caused by mutations in a HOX gene.[24] The evolutionarily highly conserved homeobox HOX genes encode transcription factors that play key roles in embryonic development, including growth and patterning of the vertebrate limb.[25] The wild-type HOXD13 contains 15 alanine residues near its N-terminus, while in synpolydactyly patients the alanine stretch is expanded to 22-29 residues.[24,26]

Polyalanine stretch expansion in the transcription factor CBFA1 causes another abnormality of bone formation: Cleidocranial dysplasia.[27] Cleidocranial dysplasia is a disorder exhibiting defective endochondral and intramembranous bone formation.[28] The typical features include hypoplasia/aplasia of clavicles, patent fontanelles, Wormian bones (additional cranial plates caused by abnormal ossification of the calvaria), supernumerary teeth, short stature, and other skeletal changes.[29,30] CBFA1 is a transcription factor in the runt family and is essential for osteoblastic differentiation.[31] The human CBFA1 contains 17 alanines in the N-terminus and expansion in this stretch to 27 alanines causes cleidocranial dysplasia.[27]

Holoprosencephaly is a common developmental anomaly of the forebrain and midface in which the cerebral hemispheres fail to separate into distinct left and right halves.[32] Genetic studies showed that heterozygous mutations in the ZIC2 gene are associated with holoprosencephaly.[33] ZIC proteins are transcription factors and are differentially expressed during the early development.[34] The expression of ZIC2 is limited to the cerebellum.[33] The polyalanine stretch expansion from 15 to 25 in the C-terminus of ZIC2 causes holoprosencephaly.[33]

Hand-foot-genital syndrome is a rare dominantly inherited condition characterized by distal limb and distal genitourinary tract malformations.[35] Hand-foot-genital syndrome is reported to be associated with HOXA13 gene mutations.[36] An expansion of a polyalanine stretch from 15 to 22-23 alanines in the N-terminus of HOXA13 results in a phenotype of hand-foot-genital syndrome. HOXA13 is the second HOX gene in which a polyalanine stretch expansion causes bone malformation.

It has recently been shown that polyalanine expansion from 14 to 24 in FOXL2 protein causes type II blepharophimosis/ptosis/epicanthus inversus syndrome (BPES).[37] Unlike type I BPES that is associated with ovarian failure, type II affected individuals are fertile. Both types of BPES are autosomal dominant conditions characterized by eyelid dysplasia, namely small palpebral fissures (blepharophimosis), drooping eyelids (ptosis), and a tiny skin fold running inward and upward from the lower lid (epicanthus inversus).[38] FOXL2 is a member of the winged helix/forkhead family of transcription factors. Forkhead proteins are found in all eukaryotes and play important roles in the establishment of the body axis and the development of tissues from all three germ layers in animals.[39]

Compared with HOXD13, CBFA1, ZIC2, HOXA13, and FOXL2 proteins, PABPN1 is structurally and functionally different in that: 1) PABPN1 is an RNA binding protein that is involved in 3'-end processing, while the other proteins are transcription factors; 2) the wild-type PABPN1 contains a stretch of 10 alanines and an expansion of 10 alanines to 12 causes dominant OPMD, whereas the other five proteins contain stretches ranging from 14 to 17 alanines and require expansions to 22 alanines or more to cause diseases; 3) PABPN1 is a small protein with 306 amino acids while the other five are relatively bigger with length ranging from 343 to 533; 4) polyalanine expansion of PABPN1 induces intranuclear inclusions and

https://doi.org/10.1017/S0317167100002365 Published online by Cambridge University Press

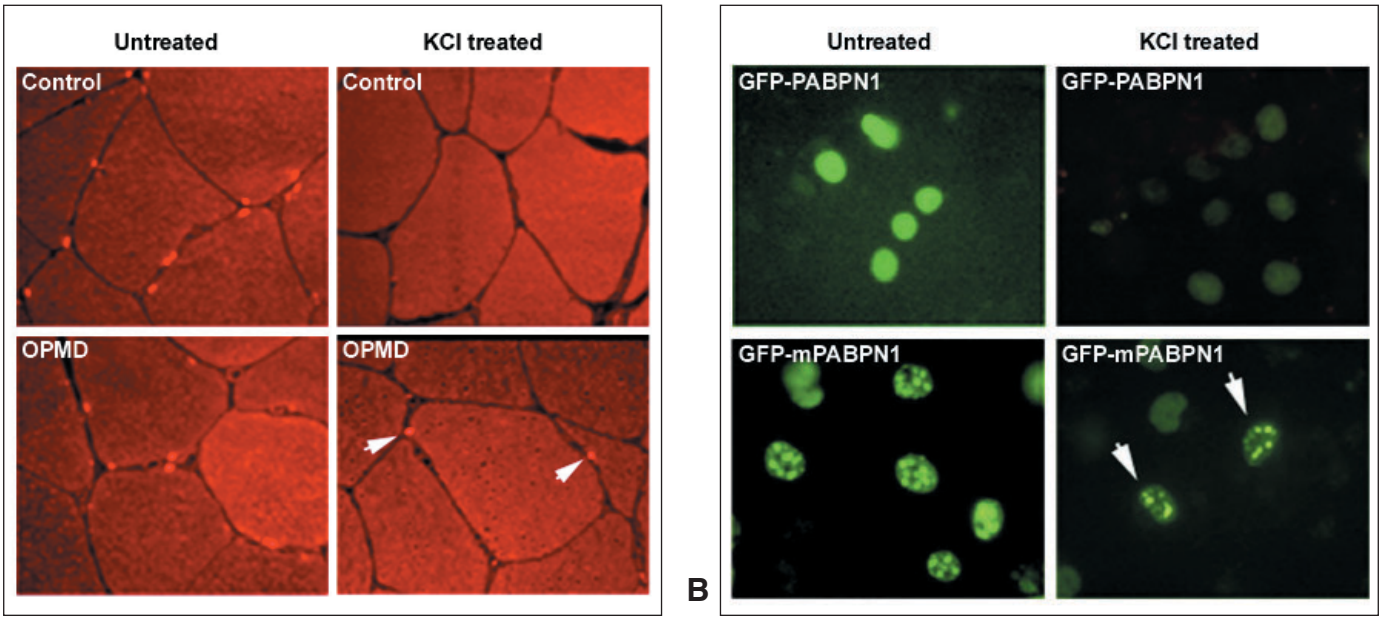

Figure 1: *A. Insoluble OPMD intranuclear inclusions. Immunohistochemistry was performed on the cross-sections of deltoid muscle from a control subject and an OPMD patient. Sections were either immunostained without KCl treatment (left panels) or treated with 1M KCl for 5 min at room temperature before immunostaining to remove the soluble proteins (right panels). Immunohistochemistry was done using polyclonal anti-PABPN1 antibody and rhodamine conjugated secondary antibody. PABPN1 in the control subject is soluble and not resistant to KCl treatment while the one in OPMD inclusions is insoluble and resistant to KCl extraction. Arrowheads indicate the positively stained insoluble intranuclear inclusions. B. Expression of mPABPN1 induces formation of insoluble intranuclear aggregates. The green fluorescent protein (GFP) was fused to PABPN1 (wild-type with 10 alanines) and mPABPN1 (with 17 alanines). GFP fusion proteins were transiently expressed in COS-7 cells. Three days post-transfection, the transfected cells were either fixed (left panels) or treated with KCl to remove the soluble protein before fixation (right panels). GFP fusion proteins were detected under fluorescent microscope. The wild-type PABPN1 is soluble whereas mPABPN1 induced aggregates are insoluble.*

causes muscular dystrophy, whereas polyalanine expansions in the five transcription factors are associated with developmental anomalies such as bone malformations and there is so far no protein aggregation reported. These diversities imply that the pathological mechanism of OPMD may differ from that of the other five disorders.

Polyalanine was reported to be able to induce subcellular protein aggregation.[40] When GFP is fused to different-sized polyalanine stretches and expressed in COS cells, GFP-19 alanines or more can induce the protein aggregation that is associated with cell death, while less than 19 alanine residues cannot.[40] Additional evidence of polyalanine toxicity comes from the study on Machado-Joseph disease, one of the disorders caused by the expansion of a coding CAG repeat. The CAG tract of the Machado-Joseph disease-1 gene was reported to be prone to frameshifts.[41] The transcriptional or translational frameshifts that occurred within expanded CAG tracts result in the production of both polyglutamine and polyalanine-containing mutant proteins. These polyalanine-containing proteins are present in intranuclear aggregates and may be harmful to transfected COS cells.[41] Hydrophobic polyalanine-based peptides were found to undergo conformational conversions leading to the formation of β-pleated-sheet complexes structurally similar to the polyglutamine aggregates found in neurodegenerative disorders.[42] Polyalanine oligomers are also resistant to chemical denaturation and enzymatic degradation.[43] These physical characteristics of polyalanine stretches might play a role in the formation of protein aggregates.

Polyalanine expanded PABPN1 induces the formation of insoluble intranuclear protein aggregation

Since Tome and Fardeau[4] identified the unique filamentous intranuclear inclusions in the deltoid muscle of three unrelated OPMD patients in 1980, intranuclear inclusions have been considered a specific pathological marker of this disorder. Patient studies showed that OPMD intranuclear inclusions can be labelled with antibodies against PABPN1, ubiquitin, and subunits of the proteasome.[44] The intranuclear inclusions in OPMD patients are insoluble and resistant to KCl extraction (Figure 1A), which is used to dissolve soluble protein aggregates.[44] Expression of mPABPN1 with 17 alanines in COS-7 cells induces formation of large intranuclear protein aggregation three days after transfection (Figure 1B).[45] Expression of mPABPN1 with 13 alanines also induces large intranuclear protein aggregation.[46] Intranuclear inclusions induced by mPABPN1 are also insoluble and resistant to KCl treatment (Figure 1B).[47] Electron microscopy studies showed that the filamentous intranuclear protein aggregates induced by over-expression of mPABPN1 in cell lines are morphologically similar to the intranuclear inclusions observed in OPMD muscle nuclei (Bernard Brais, unpublished observations). These

https://doi.org/10.1017/S0317167100002365 Published online by Cambridge University Press

observations provide biochemical evidence that polyalanine expanded PABPN1 induces intranuclear protein aggregation and causes OPMD.

OPMD INTRANUCLEAR INCLUSIONS ARE ASSOCIATED WITH CELL DEATH

The polyalanine stretches in the wild-type transcription factors described above range from 14 to 17 alanines and do not induce protein aggregation. To induce protein aggregation and cause cell death require a stretch of at least 19 alanines.[44] However, the polyalanine stretch expansion from 10 to 12 alanines in PABPN1 induces protein aggregation and causes dominant OPMD.[13] One possible explanation for this paradox may be due to the small size of PABPN1. Hydrophobic alanine stretches are usually buried inside the molecule. Expansion of the alanine stretch might expose the hydrophobic region, enable the protein to perform self-association through the exposed hydrophobic regions, and induce protein aggregation. PABPN1 is less able to bury the expanded hydrophobic polyalanine stretch due to its small size. However, this model does not explain why polyalanine expansions of small proteins like HOXD13 with 15 alanines do not result in protein aggregation. This paradox may be explained in part by the demonstration that oligomerization of mPABPN1 facilitates intranuclear protein aggregation.[47]

PABPN1, when bound to the poly(A) tail of mRNA, forms both linear filaments and discrete-sized, compact oligomeric particles *in vitro*,[21] and also form oligomers in the absence of mRNA.[22] Oligomerization of PABPN1 is mediated by two partially overlapping potential oliogmerization domains. These two domains are located among AA155-306 in the C-terminal region, far from the polyalanine stretch residing from AA2-11. Expansion of the polyalanine stretch does not affect the oligomerization of this protein. Inactivating oligomerization of mPABPN1 by deletions in either of the oligomerization domains prevents the formation of intranuclear protein aggregation. These observations lead to a conclusion that oligomerization of mPABPN1 facilitates intranuclear protein aggregation induced by mPABPN1, while the expanded alanine stretch in PABPN1 is necessary but not sufficient to induce OPMD protein aggregation.[47]

The presence of abnormal protein aggregates is a relatively common finding in a number of neurodegenerative diseases.[48,49] The best characterized and most common of such disorders is Huntington's disease that results from an expansion of the polyglutamine stretch in the huntingtin protein, leading to its aggregation and abnormal association with various proteins.[50] Eventually, the impaired neuronal function becomes evident and may trigger cell death.[51] It has been widely assumed that aggregate formation is a critical event triggering neuropathology in this disease.[49] However, there is also appreciable evidence against this view. Deletion of the C-terminal portion of huntingtin enhances the formation of inclusions but does not increase the fraction of apoptotic cells.[52] Furthermore, in spinocerebellar ataxia type 1 (SCA1), deletion of the self-association regions of ataxin-1 protein blocks aggregation but does not suppress apoptosis.[53] In OPMD pathogenesis studies, a key question is whether OPMD intranuclear inclusions are toxic. Current data suggest that the intranuclear inclusions induced by mPABPN1 are associated with cell death.[47,54] Cells expressing mPABPN1 with 17 alanines die much earlier than the ones expressing the normal PABPN1. Inactivating oligomerization of mPABPN1 with 17 alanines prevents the formation of intranuclear protein aggregation and reduces cell death. Co-expressing chaperone proteins with mPABPN1, Bao et al[54] recently found that the formation of aggregates of mPABPN1 is associated with cell death, while expression of chaperones can reduce aggregation of mPABPN1 and reduce cell death. These observations indicate that mPABPN1 induced protein aggregation is likely to be a critical event in triggering OPMD pathogenesis. Cell death induced by expression of mPABPN1 seems not through apoptosis. Brais et al recently found that apoptotic caspases 1, 6, 8, and 9 are not activated, apoptosis staining (TUNEL) is insignificant, PARP protein cleavage is not detectable, and apoptotic morphology cannot be observed (Bernard Brais, unpublished observations). It is possible that cell death in OPMD may be caused by a non-apoptotic mechanism as reported in a SCA3 cellular model and a Huntington's disease transgenic model.[55,56]

POTENTIAL EVIDENCE OF OPMD INCLUSION TOXICITY

1. OPMD intranuclear inclusions sequester poly(A) RNA in the nucleus

Having found that mPABPN1 induces the formation of insoluble intranuclear inclusions that are toxic and associated with cell death, a key question is how OPMD aggregates cause cell death. PABPN1 stimulates polyadenylation, controls the length of the RNA poly(A) tail, and coats the poly(A) tail in the nucleus.[20,57] Polyalanine stretch expansion in PABPN1 might interfere with its normal functions and impair poly(A) tail synthesis. However, patient studies showed that poly(A) tail length distribution in cultured myoblasts from a homozygous OPMD patient is not different from that in normal cells,[44] suggesting that polyalanine expanded PABPN1 might maintain the polyadenylation function. Recent evidence indicates that PABPN1 may have an additional role in export of mRNA from the nucleus to the cytoplasm.[58,59] Oculopharyngeal muscular dystrophy patient studies showed that the intranuclear inclusions consist of PABPN1 and poly(A) RNA.[44] The presence of poly(A) RNA in the OPMD intranuclear inclusions may represent mRNA sequestered by the insoluble PABPN1 aggregates. This raises the possibility that OPMD nuclear inclusions might be "mRNA traps" that prevent mRNA export from the nucleus to the cytoplasm. Interfering with mRNA export may be detrimental to cells and contribute to cell death.

2. OPMD intranuclear inclusions sequester hnRNP A1

Ideally direct analysis of the intranuclear inclusions is required to understand why OPMD inclusions are toxic. However, due to difficulties in separating and purifying the OPMD inclusions, direct biochemical analyses are hard to pursue. Using a yeast two-hybrid library screen, we recently found that C-terminus of PABPN1 interacts with hnRNP A1 and hnRNP A/B, and showed that hnRNP A1 is sequestered in OPMD intranuclear inclusions (unpublished observations). The hnRNP proteins are predominantly nuclear RNA-binding proteins associated specifically with pre-mRNA and mRNA molecules.[60,61] The nucleocytoplasmic shuttling hnRNP A1 has been found to be involved in mRNA export.[61-64] PABPN1 is a

https://doi.org/10.1017/S0317167100002365 Published online by Cambridge University Press

shuttling protein, coats the poly(A) tail of mRNA in the nucleus, and is involved in mRNA export.[44] Identification of hnRNP A1 in OPMD intranuclear aggregates supports the notion that OPMD aggregates might interfere with mRNA export. In addition, hnRNP A1 protein is also involved in pre-mRNA splicing, telomere length maintenance, transcription and translation regulations, pre-mRNA 3'-end processing, and mRNA stability.[65] Sequestering some hnRNP A1 molecules in the OPMD aggregates might reduce the normal concentration of this protein in the nucleus and affect its functions. It could also be detrimental to the cell if, to carry out its normal function, hnRNP A1 needs its binding partner PABPN1 that is largely aggregated in the OPMD inclusions.

3. OPMD intranuclear inclusions sequester hnRNP A/B

We also found that hnRNP A/B interacts with PABPN1 and is sequestered in OPMD intranuclear inclusions (unpublished observations). This protein was first purified as an RNA binding protein and named C-type hnRNP protein.[66] It was subsequently cloned and renamed to type A/B hnRNP protein due to its homologous structure to hnRNP A and B proteins.[67] The binding of this protein to RNA disrupts the residual secondary structure of RNA.[67] Its isoform was cloned in 1997 and named ABBP-1.[68] ABBP-1 is able to interact with apolipoprotein B (apoB) mRNA and is involved in apoB pre-mRNA editing, a post-transcriptional mRNA modification process in mammals.[68] It consists of a C to U conversion of the codon CAA, encoding glutamine 2153, to UAA, an in-frame stop codon in apoB mRNA, resulting in a production of an isoform apoB protein.[69] The process requires the presence of the poly(A) tail of apoB mRNA.[70] Since the poly(A) tail of mRNA is always coated by PABPN1 molecules in the nucleus, the interaction between ABBP-1 and PABPN1 implies that PABPN1 might be involved in apoB mRNA editing. Aggregation of mPABPN1 might interfere with the normal function of hnRNP A/B that requires its binding partner PABPN1 to carry out its functions. Sequestering hnRNP A/B protein in OPMD intranuclear inclusions suggests that OPMD inclusions might interfere with mRNA editing and trap pre-mRNA.

4. OPMD inclusions sequester a nuclear protein, Ski-interacting protein (SKIP)

PABPN1 is expressed ubiquitously, but the clinical and pathological features of OPMD patients are mainly restricted to the skeletal muscle. This raises the possibility that PABPN1 plays a specific role in skeletal muscle cells. Recent evidence showed that over-expression of PABPN1 in a muscle cell line enhances myotube formation accompanied by an increased expression of myogenic factors, MyoD and myogenin.[46] PABPN1 was also found to interact with SKIP,[46] a potential co-factor of transcription, originally identified as a protein that interacts with both the cellular and viral forms of the oncoprotein Ski.[71] Both SKIP and PABPN1 can interact with MyoD to form a single complex, suggesting that PABPN1 and SKIP might directly control the expression of muscle-specific genes at the transcriptional level. Co-expressing SKIP with mPABPN1 in cells, SKIP was positively detected in the OPMD intranuclear aggregates.[46] Sequestering of SKIP in the OPMD intranuclear aggregates might interfere with muscle specific gene transcription. Why and how muscle cells are more susceptible to mutations in PABPN1 than other types of cells remains unknown.

A PROPOSED POTENTIAL MOLECULAR MECHANISM FOR OPMD PATHOGENESIS

Since the identification of the OPMD gene, many studies have begun to shed new light on the pathogenesis of this disease. Based on the previous studies and information available, we propose a potential molecular mechanism for OPMD pathogenesis (Figure 2). Expansion of polyalanine stretch in PABPN1 causes misfolding or unfolding and exposes its hydrophobic alanine stretch that would otherwise be buried inside in the wild type form. The longer the polyalanine stretch is, the more exposed the hydrophobic region is. Misfolded mPABPN1 therefore acquires a gain-of-function that enables it to perform weak self-association through the exposed hydrophobic regions. Because the polyalanine stretch is located at the N-terminus, far from both oligomerization domains, the expanded polyalanine stretch does not interfere with its oligomerization. When mPABPN1 performs weak self-association, oligomerization of mPABPN1 facilitates this weak interaction by linking mPABPN1 molecules together to promote inclusion formation. Concurrently, mPABPN1 is detected and

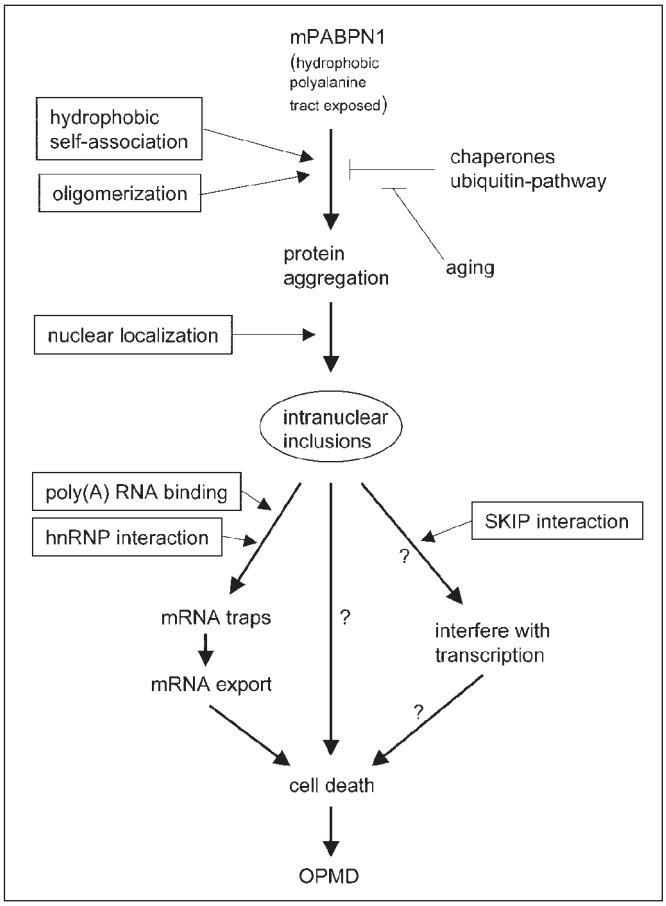

Figure 2: *A proposed potential molecular mechanism for OPMD pathogenesis.*

https://doi.org/10.1017/S0317167100002365 Published online by Cambridge University Press

bound by chaperones, components of the ubiquitin-proteasome pathway, as well as other crucial proteins. Binding of these proteins to mPABPN1 may promote refolding, solubilization, and/or degradation of mPABPN1. Essentially, there is a balance between mPABPN1 elimination and aggregation. Upon aging of the cell, its ability to process abnormal proteins may decrease, therefore resulting in a tilt in balance favoring protein aggregation. Because PABPN1 coats the poly(A) tail of mRNA and interacts with hnRNP proteins that are involved in mRNA processing and export, OPMD intranuclear protein aggregates might sequester mRNA and hnRNPs, impair mRNA processing, and interfere with mRNA nucleocytoplasmic export. The aggregates also sequester co-transcription factor SKIP and might interfere with muscle-specific gene expression. Furthermore, the presence of huge intranuclear aggregates might also interfere with functions of the nucleus. Under such conditions, muscle cells cannot perform their normal function and subsequently undergo cell death. Gradual accumulative muscle cell death results in OPMD phenotype.

Comments

It is becoming increasingly clear that, although the expanded polyalanine stretch in mPABPN1 is crucial to initiate OPMD pathogenesis, residues outside the polyalanine stretch also play important roles in the disease. These residues participate in important cellular processes such as oligomerization, nuclear localization, and its interaction with other molecules that very likely have impact on OPMD progression. To further understand OPMD pathogenesis, there are still many important questions to answer. Firstly, whether nuclear localization of OPMD inclusions is required for this disease progression. In spinocerebellar ataxia type 3, nuclear localization of the polyglutamine expanded ataxin-1 protein is critical to initiate pathogenesis: transgenic mice carrying the mutated ataxin-1 with a mutated nuclear localization signal do not develop disease.[53] Finding the relationship between subcellular location of OPMD inclusions and their toxicity is critical to understand the pathogenesis of this disease. Secondly, further characterizing the mRNA binding activity of mPABPN1 is necessary to confirm if OPMD intranuclear inclusions trap mRNA and interfere with mRNA export. It is of interest to find out whether inactivating the mRNA binding activity of mPABPN1 will have any impact on OPMD pathogenesis. Thirdly, why do mutations in a ubiquitous protein PABPN1 induce intranuclear inclusions mainly in muscle cells? Further studies on muscle specific functions of PABPN1 and whether muscle specific proteins are involved in OPMD pathogenesis are required to elucidate the pathological mechanism of this disease. Finally, why is OPMD adult-onset? Oculopharyngeal muscular dystrophy transgenic animal models are needed to define the time-frame between appearance of intranuclear inclusions and the first sign of OPMD phenotype. Oculopharyngeal muscular dystrophy animal models will also be useful to study whether aging is playing a role in initiating OPMD symptoms.

Acknowledgements

We thank Dr Bernard Brais for sharing unpublished information; Janet Laganiere and Dr Patrick Dion for providing the OPMD nuclear inclusion figure. We thank Dr Claudia Gaspar for careful reading and comments on the manuscript.

This work was supported by the Muscular Dystrophy Association (USA) and the Federation Foundation of Greater Philadelphia. Guy A. Rouleau is supported by the CIHR.

References

1. Brais B, Xie YG, Sanson M, et al. The oculopharyngeal muscular dystrophy locus maps to the region of the cardiac alpha and beta myosin heavy chain genes on chromosome 14q11.2-q13. Hum Mol Genet 1995; 4: 429-434.
2. Brunet G, Tome FM, Samson F, et al. Dytrophie musculaire oculopharyngee. Recensement des familles francaises et etude genealogique. Rev Neurol 1990; 146: 425-429.
3. Brais B, Rouleau GA, Bouchard JP, et al. Oculopharyngeal muscular dystrophy. Semin Neurol 1999; 19: 59-66.
4. Tome FM, Fardeau M. Nuclear inclusions in oculopharyngeal dystrophy. Acta Neuropathologica 1980; 49: 85-87.
5. Pratt MF, Meyers PK. Oculopharyngeal muscular dystrophy: recent ultrastructural evidence for mitochondrial abnormalities. Laryngoscope 1986; 96: 368-373.
6. Tome FM, Askanas V, Engel WK, et al. Nuclear inclusions in innervated cultured muscle fibers from patients with oculopharyngeal muscular dystrophy. Neurol 1989; 39: 926-932.
7. Bouchard JP, Gagne F, Tome FM, Brunet D. Nuclear inclusions in oculopharyngeal muscular dystrophy in Quebec. Can J Neurol Sci 1989; 16: 446-450.
8. Coquet M, Vital C, Julien J. Presence of inclusion body myositis-like filaments in oculopharyngeal muscular dystrophy. Ultrastructural study of 10 cases. Neuropathol Appl Neurobiol 1990; 16: 393-400.
9. Blumen SC, Sadeh M, Korczyn AD, et al. Intranuclear inclusions in oculopharyngeal muscular dystrophy among Bukhara Jews. Neurol 1996; 46: 1324-1328.
10. Stajich JM, Gilchrist JM, Lennon F, et al. Confirmation of linkage of oculopharyngeal muscular dystrophy to chromosome 14q11.2-q13 in American families suggests the existence of a second causal mutation. Neuromuscul Disord 1997; 7(Suppl 1): S75-S81.
11. Teh BT, Sullivan AA, Farnebo F, et al. Oculopharyngeal muscular dystrophy (OPMD)-report and genetic studies of an Australian kindred. Clin Genet 1997; 51: 52-55.
12. Porschke H, Kress W, Reichmann H, et al. Oculopharyngeal muscular dystrophy in a northern German family linked to chromosome 14q, and presenting carnitine deficiency. Neuromuscul Disord 1997; 7(Suppl 1): S57-S62.
13. Brais B, Bouchard JP, Xie YG, et al. Short GCG expansions in the PABP2 gene cause oculopharyngeal muscular dystrophy. Nat Genet 1998; 18: 164-167.
14. Wahle E. A novel poly(A)-binding protein acts as a specificity factor in the second phase of messenger RNA polyadenylation. Cell 1991; 66: 759-768.
15. Colgan DF, Manley JL. Mechanism and regulation of mRNA polyadenylation. Genes Dev 1997; 11: 2755-2766.
16. Minvielle-Sebastia L, Keller W. mRNA polyadenylation and its coupling to other RNA processing reactions and to transcription. Curr Opin Cell Biol 1999; 11: 352-357.
17. Barabino SML, Keller W. Last but not least: regulated poly(A) tail formation. Cell 1999; 99: 9-11.
18. Wahle E. Poly(A) tail length control is caused by termination of processive synthesis. J Biol Chem 1995; 270: 2800-2808.
19. Bienroth S, Keller W, Wahle E. Assembly of a processive messenger RNA polyadenylation complex. EMBO J 1993; 12: 585-594.
20. Wahle E, Lustig A, Jeno P, Maurer P. Mammalian poly(A)-binding protein II. Physical properties and binding to polynucleotides. J Biol Chem 1993; 268: 2937-2945.
21. Keller RW, Kuhn U, Aragon M, et al. The nuclear poly(A) binding protein, PABP2, forms an oligomeric particle covering the length of the poly(A) tail. J Mol Biol 2000; 297: 569-583.
22. Nemeth A, Krause S, Blank D, et al. Isolation of genomic and cDNA clones encoding bovine poly(A) binding protein II. Nuclei Acid Res 1995; 23: 4034-4041.

23. Temtamy SA, McKusick VA. Syndactyly as an isolated malformation. In: The Genetics of Hand Malformations. Birth Defects: Original Article Series vol. XIV (3). Alan RL. New York, 1978; 301-322.
24. Muragaki Y, Mundlos S, Upton J, Olsen BR. Altered growth and branching patterns in synpolydactyly caused by mutations in HOXD13. Science 1996; 272: 548-551.
25. Izpisua-Belmonte JC, Duboule D. Homeobox genes and pattern formation in the vertebrate limb. Dev Biol 1992; 152: 26-36.
26. Goodman FR, Mundlos S, Muragaki Y, et al. Synpolydactyly phenotypes correlated with size of expansions in HOXD13 polyalanine tract. Proc Natl Acad Sci USA 1997; 94: 7458-7463.
27. Mundlos S, Otto F, Mundlos C, et al. Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. Cell 1997; 89: 773-779.
28. Jones KL. Smith's Recognizable Patterns of Human Malformation, 5th ed. 1997 Philadelphia: W.B. Saunders Company.
29. Mundlos S, Olsen BR. Heritable diseases of the skeleton. Part I: molecular insights into skeletal development-transcription factors and signaling pathways. FASEB J 1997; 11: 125-132.
30. Mundlos S, Olsen BR. Heritable diseases of the skeleton. Part II: molecular insights into skeletal development-matrix components and their homeostasis. FASEB J 1997; 11: 227-233.
31. Otto F, Thornell AP, Crompton T, et al. Cbfa1, a candidate gene for Cleidocranial Dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell 1997; 89: 765-771.
32. Nanni L, Croen LA, Lammer EJ, Muenke M. Holoprosencephaly: molecular study of a California population. Am J Med Genet 2000; 90: 315-319.
33. Brown SA, Warburton D, Brown LY, et al. Holoprosencephaly due to mutations in ZIC2, a homologue of Drosophila odd-paired. Nat Genet 1998; 20: 180-183.
34. Nagai T, Aruga J, Takada S, et al. The expressions of the mouse Zic1, Zic2, and Zic3 genes suggest an essential role for Zic genes in body pattern formation. Dev Biol 1997; 182: 299-313.
35. Halal F. The hand-foot-genital (hand-foot-uterus) syndrome: family report and update. Am J Med Genet 1988; 30: 793-803.
36. Goodman FR, Bacchelli C, Brady AF, et al. Novel HOXA13 mutations and the phenotypic spectrum of hand-foot-genital syndrome. Am J Hum Genet 2000; 67: 197-202.
37. Crisponi L, Deiana M, Loi A, et al. The putative forkhead transcription factor FOXL2 is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. Nat Genet 2001; 27: 159-166.
38. Zlotogora J, Sagi M, Cohen T. The blepharophimosis, ptosis, and epicanthus inversus syndrome: delineation of two types. Am J Hum Genet 1983; 35: 1020-1027.
39. Prueitt RL, Zinn AR. A fork in the road to fertility. Nat Genet 2001; 27: 132-134.
40. Rankin J, Wyttenbach A, Rubinsztein DC. Intracellular green fluorescent protein-polyalanine aggregates are associated with cell death. Biochem J 2000; 348: 15-19.
41. Gaspar C, Jannatipour M, Dion P, et al. CAG tract of MJD-1 may be prone to frameshifts causing polyalanine accumulation. Hum Mol Genet 2000; 9: 1957-1966.
42. Blondelle SE, Forood B, Houghten RA, Perez-Paya E. Polyalanine-based peptides as models for self-associated beta-pleated-sheet complexes. Biochem 1997; 36: 8393-8400.
43. Forood B, Perez-Paya E, Houghten RA, Blondelle SE. Formation of an extremely stable polyalanine beta-sheet macromolecule. Biochem Biophys Res Commun 1995; 211: 7-13.
44. Calado A, Tome FMS, Brais B, et al. Nuclear inclusions in oculopharyngeal muscular dystrophy consist of poly(A) binding protein 2 aggregates which sequester poly(A) RNA. Hum Mol Genet 2000; 9: 2321-2328.
45. Shanmugam V, Dion P, Rochefort D, et al. PABP2 polyalanine tract expansion causes intranuclear inclusions in oculopharyngeal muscular dystrophy. Ann Neurol 2000; 48: 798-802.
46. Kim YJ, Noguchi S, Hayashi YK, et al. The product of an oculopharyngeal muscular dystrophy gene, poly(A)-binding protein 2, interacts with SKIP and stimulates muscle-specific gene expression. Hum Mol Genet 2001; 10: 1129-1139.
47. Fan X, Dion P, Laganiere J, et al. Oligomerization of polyalanine expanded PABPN1 facilitates nuclear protein aggregation that is associated with cell death. Hum Mol Genet 2001; 10: 2341-2351.
48. Ferrigno P, Silver PA. Polyglutamine expansions: proteolysis, chaperones, and the dangers of promiscuity. Neuron 2000; 26: 9-12.
49. Sherman MY, Goldberg AL. Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. Neuron 2001; 29: 15-32.
50. Bates GP, Mangiarini L, Davies SW. Transgenic mice in the study of polyglutamine repeat expansion diseases. Brain Pathol 1998; 8: 699-714.
51. Yong A. Huntington's Disease and Other Trinucleotide Repeat Disorders. Martin J, Eds. New York: Scientific American, Inc. 1998.
52. Saudou F, Finkbeiner S, Devys D, Greenberg ME. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 1998; 95: 55-66.
53. Klement IA, Skinner PJ, Kaytor MD, et al. Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. Cell 1998; 95: 41-53.
54. Bao YP, Cook LJ, O'Donovan D, et al. Mammalian, yeast, bacterial, and chemical chaperones reduce aggregate formation and death in a cell model of oculopharyngeal muscular dystrophy. J Biol Chem 2002; 277: 12263-12269.
55. Evert BO, Wullner U, Schulz JB, et al. High level expression of expanded full-length ataxin-3 in vitro causes cell death and formation of intranuclear inclusions in neuronal cells. Hum Mol Genet 1999; 8: 1169-1176.
56. Turmaine M, Raza A, Mahal A, et al. Nonapoptotic neurodegeneration in a transgenic mouse model of Huntington's disease. Proc Natl Acad Sci USA 2000; 97: 8089-8097.
57. Wahle E, Kuhn U. The mechanism of 3' cleavage and polyadenylation of eukaryotic pre-mRNA. Prog Nucleic Acid Res Mol Biol 1997; 57: 41-71.
58. Chen Z, Li Y, Krug R. Influenza A virus NS1 protein targets poly(A)-binding protein II of the cellular 3'-end processing machinery. EMBO J 1999; 18: 2273-2283.
59. Calado A, Kutay U, Kuhn U, et al. Deciphering the cellular pathway for transport of poly(A)-binding protein II. RNA 2000; 6: 245-256.
60. Dreyfuss G, Matunis MJ, Pino-Roma S, Burd CG. hnRNP proteins and the biogenesis of mRNA. Ann Rev Biochem 1993; 62: 289-321.
61. Swanson MS. Functions of nuclear pre-mRNA/mRNA binding proteins. In: Lamond AI, Eds. Pre-mRNA Processing. Berlin/New York: Springer Verlag, 1995: 17-33.
62. Pinol-Roma S, Dreyfuss G. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. Nature 1992; 355: 730-732.
63. Visa N, Alzhanova-Ericsson AT, Sun X, Kiseleva E. A pre-mRNA-binding protein accompanies the RNA from the gene through the nuclear pores and into polysomes. Cell 1996; 84: 253-264.
64. Nakielny S, Dreyfuss G. Nuclear export of proteins and RNAs. Curr Opin Cell Biol 1997; 9: 420-429.
65. Krecic AM, Swanson MS. hnRNP complexes: composition, structure, and function. Curr Opin Cell Biol 1999; 11: 363-371.
66. Kumar A, Sierakowska H, Szer W. Purification and RNA binding properties of a C-type hnRNP protein from HeLa cells. J Biol Chem 1987; 262: 17126-17137.
67. Khan FA, Jaiswal AK, Szer W. Cloning and sequence analysis of a human type A/B hnRNP protein. FEBS 1991; 290: 159-161.
68. Lau PP, Zhu HJ, Nakamuta M, Chan L. Cloning of an apobec-1-binding protein that also interacts with apolipoprotein B mRNA and evidence for its involvement in RNA editing. J Biol Chem 1997; 272: 1452-1455.
69. Innerarity TL, Boren J, Yamanaka S, Olofsson SO. Biosynthesis of apolipoprotein B48-containing lipoproteins. J Biol Chem 1996; 271: 2353-2356.
70. Lau PP, Xiong W, Zhu HJ, et al. Apolipoprotein B mRNA editing is an intranuclear event that occurs posttranscriptionally coincident with splicing and polyadenylation. J Biol Chem 1991; 266: 20550-20554.
71. Dahl R, Wani B, Hayman MJ. The Ski oncoprotein interacts with Skip, the human homolog of Drosopila Bx42. Oncogene 1998; 16: 1579-1586.