AUTOPHAGY IN SKELETAL MUSCLE: AUTOPHAGIC VACUOLAR MYOPATHIES

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Received February 16, 2005

Abstract : Eukaryotic cells from yeast to human primarily use two distinct major mechanism for intracellular degradation/recycling system: the autophagy and proteasome. Autophagy contributes to the turnover of cellular components by delivering portions of the cytoplasm and organelles to lysosomes, where they are digested. Moreover, autophagy is involved in programmed cell death (PCD) and is called type II PCD, which differs from apoptosis (type I PCD). By mostly morphological studies, autophagy has been linked to disease processes: cancer, liver diseases, neurodegeneration, muscular disorders, and so on. Actually, lysosomes or autophagic vacuoles are morphologically unremarkable in normal muscle. However, in certain muscular disorders, the lysosomal system becomes prominent, indicating that autophagy is essential for muscle fibers. Here, we described current knowledge about the role of autophagy and the autophagy in muscular disorders: autophagic vacuolar myopathies.

Key words : autophagy, autophagic vacuole, lysosome, Danon disease, rimmed vacuole

I. ROLE OF AUTOPHAGY

Life can only be established based on a homeostatic balance between synthesis and degradation. For turnover of cellular components, eukaryotic cells are equipped with several degradation systems, one of which is the process of autophagy.[1,2] Autophagy is a transport pathway leading from the cytoplasm to lysosomes.[3] Proteasome generally serves to selectively degrade short-lived proteins, while autophagy serves to degrade most long-lived proteins, which constitute the majority of cellular material, in lysosomes.

In the autophagic process, the isolation membrane, which is a single-membrane structure, first surrounds portions of the cytoplasm and organelles (Fig. 1). Fusion of the tips of the isolation membrane to each other forms a double-membrane spherical autophagosome. After the autophagosome fuses with lysosomes and the sequestered contents, the inner membranes are degraded by lysosomal hydrolases. The lifetime of autophagosomes is very short in contrast to other organelles. Amino acids produced by the degradation of cytosolic components can be reused by the cell. Therefore, autophagy can be considered to be an efficient recycling system. Although autophagy is usually suppressed to a basal level in most cells, it can be induced by a change of environmental conditions such as nutrient depletion.

The molecular mechanism of autophagy has entered the research spotlight largely during these past 10 years. Genetic approaches using yeast were introduced in this research field

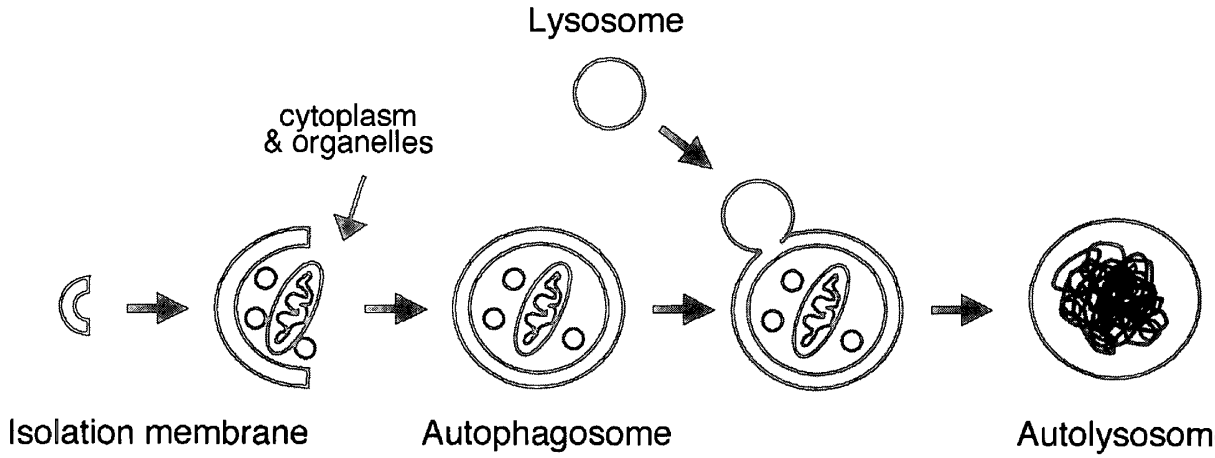

Fig. 1. Scheme of autophagy in mammalian cells. A sequestering membrane, termed an isolation membrane, forms from the pre-autophagosomal structure. The isolation membrane enwraps cytosol and organelles; on completion, a double-membrane vesicle, the autophagosome or autophagic vacuole, is formed. The autophagosome acquires hydrolytic enzymes by fusing with the lysosome to generate an autolysosome, and the inner vesicle of the autophagosome is released into the lumen and is degraded by lysosomal hydrolases.

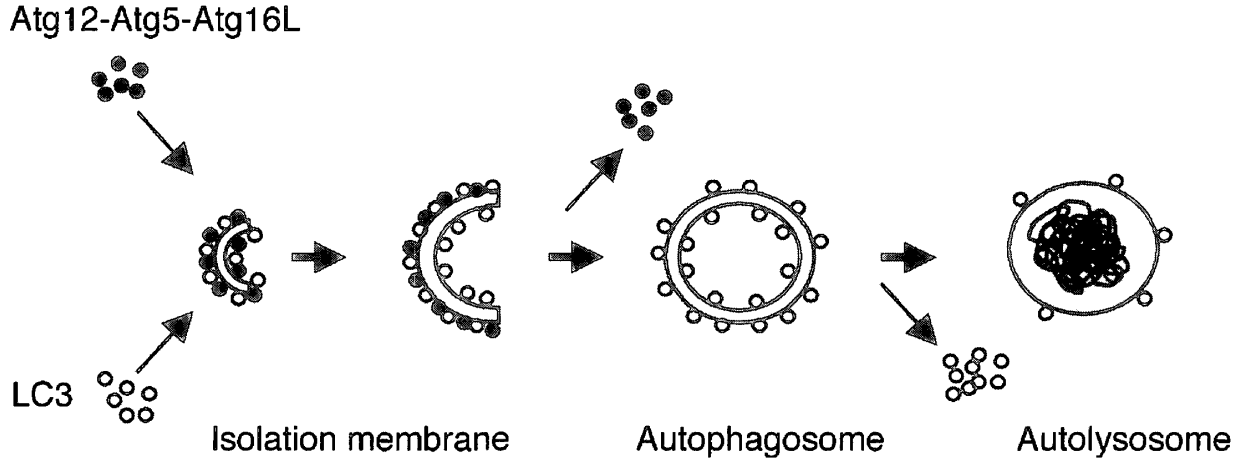

Fig. 2. Model of autophagosome formation in mammalian cells. The Atg12-Atg5 conjugate and Atg16L localize to the isolation membrane throughout its elongation process. LC3 is recruited to the membrane in the Atg5-dependent manner. Atg12-Atg5 and Atg16L dissociate from the membrane upon completion of autophagosome formation, while LC3 remains on the autophagosome membrane. LC3 is also detected on membrane autolysosomes, which have been fused with lysosomes.

and at least 16 genes required for autophagosome formation were identified such as *Atg2*, *Atg5*, *Atg12* and so on.[3] Although most Atg12-Atg5-Atg16L complex resides in the cytosol, a small fraction localizes on the isolation membrane throughout its elongation process (Fig. 2). As the membrane elongates, Atg12-Atg5-Atg16L shows asymmetric localization, with most of them associating with the outer side of the isolation membrane. Finally, Atg12-Atg5-Atg16L dissociates from the membrane upon completion of autophagosome formation. Another useful marker protein is microtubule-associated protein 1 light chain 3 (LC3). LC3 localizes on the membrane of complete spherical autophagosomes as well as on the isolation membranes. LC3 is also detected on membrane of autolysosomes, which have

been fused with lysosomes.

Autophagy most likely contributes to the turnover of cellular components at a steady state level by delivering portions of the cytoplasm and organelles to lysosomes, where they are digested. In addition, autophagy has been demonstrated to play an important role in the degradation of excess or injured organelles.[4] Although autophagy is basically a non-selective process, it may select its target in some cases, including organelle degradation.

Ⅱ. AUTOPHAGY IN DISEASES

Autophagy occurs at basal levels in most tissues and contributes to the routine turnover of cytoplasmic components. In contrast, the dramatic enhancement of autophagy can be triggered by some conditions such as starvation and hormonal stimulation. Moreover, autophagy is also involved in development, differentiation, and tissue remodeling in various organisms.[5] In addition, the increase in autophagy is a characteristic of type II programmed cell death (also known as autophagic cell death), which differs from apoptosis (type I programmed cell death).[6] Autophagy may not only be a cause of cell death, it may also precede apoptosis as a defense mechanism. Autophagy is also implicated in wide range of diverse human diseases (Table 1): cancer, neurodegeneration, muscular disorders, liver diseases and pathogen infection.[1,3] Actually, by mostly morphological studies, autophagy has been linked to disease processes.

Paradoxically, autophagy can serve to protect cells but may also contribute to cell damage. Although whether autophagy protects from or causes disease is unclear, identification of molecules involved in autophagy will aid their elucidation. Recent study has found that the disruption of the gene of Atg5, one of mammalian homologs of the yeast proteins essential for autophagy, enhances the accumulation of aggregates of unfolded proteins, which is a cause of some degenerative disorders.[2] Therefore, it is likely that autophagy takes part in protection against abnormal protein accumulations, which are toxic to cells, by helping degrade them.

Table 1. Summary of autophagy in diseases

1. Cancer
2. Neurodegeneration
Polyglutamine diseases
Alzheimer's disease
Parkinson's disease
Prion disease
3. Muscular disorders *(described in detail in Table 2 and the text)*
Acid maltase deficiency
Danon disease
Rimmed vacuolar myopathies
4. Liver diseases
α1-antitrypsin deficiency
5. Pathogen infection

Ⅲ. AUTOPHAGY IN MUSCULAR DISORDERS: AUTOPHAGIC VACUOLAR MYOPATHIES

Although many disorders associated with deregulated autophagy have been reported, most of them are observed in nonproliferative cells, such as muscle and neuronal cells, where the accumulation of damaged materials might be severe.

Actually, lysosomes or autophagic vacuoles are morphologically unremarkable in normal muscle. However, the lysosomal system becomes prominent in certain muscular disorders, indicating that autophagy is essential for muscle fibers. Autophagic vacuolar myopathies are characterized by the development of autophagic vacuoles in the muscle fibers.[7] These myopathies may be associated with abnormality of the lysosomal system and can be classified into two groups: primary and secondary lysosomal myopathies.

Primary lysosomal myopathies include 1) acid maltase deficiency (glycogen storage disease type II), and 2) autophagic vacuolar myopathies with sarcolemmal feature and acetylcholinesterase activity. Deficiency of acid maltase, a lysosomal enzyme, has been well characterized clinically, pathologically, biochemically, and genetically, and may become treatable in the near future. The diseases in the latter category are relatively rare, but appear to be genetically heterogeneous and the list of these diseases is expanding. Danon disease, the best-characterized disorder in this group, is caused by primary deficiency of a lysosomal membrane protein, LAMP-2.[8] Therefore, diseases in this category are expected to be primary lysosomal disease.

On the other hand, rimmed vacuolar myopathies are characterized by numerous rimmed vacuoles in the muscle fibers. Rimmed vacuoles are clusters of autophagic vacuoles and myeloid bodies ultrastucturally. This group includes a number of not only non-hereditary but also hereditary muscular disorders. Interestingly, none of the genes responsible for the diseases in this group encode lysosomal proteins; they all encode extralysosomal proteins. Therefore, rimmed vacuoles are thought to be formed by not primary but secondary abnormalities of lysosome, and these myopathies are most likely secondary lysosomal myopathies.

1. ACID MALTASE DEFICIENCY

Glycogen storage disease type II, which is caused by the primary deficiency of acid maltase, is the first identified primary lysosomal myopathy.[9] Acid maltase is a lysosomal enzyme involved in the degradation of glycogen. Therefore, there is deposition of undegraded glycogen both outside and within lysosomes.[10] Deficient enzymes of other glycogen storage disease are not lysosomal enzymes. Clinically, acid maltase deficiency (AMD) is classified into three groups: infantile, childhood, and adult-onset. The infantile form is also called Pompe disease. The infantile form is most severe and is characterized by progressive weakness, hypotonia, and cardiac, liver, and tongue hypertrophy. The childhood form usually starts in infancy or early childhood, presenting as a predominantly proximal myopathy with preferential involvement of respiratory muscles. The adult-onset form presents usually with weakness and atrophy of limb-girdle muscle and again with preferential involvement of respiratory muscles. AMD is an autosomal recessive disease.

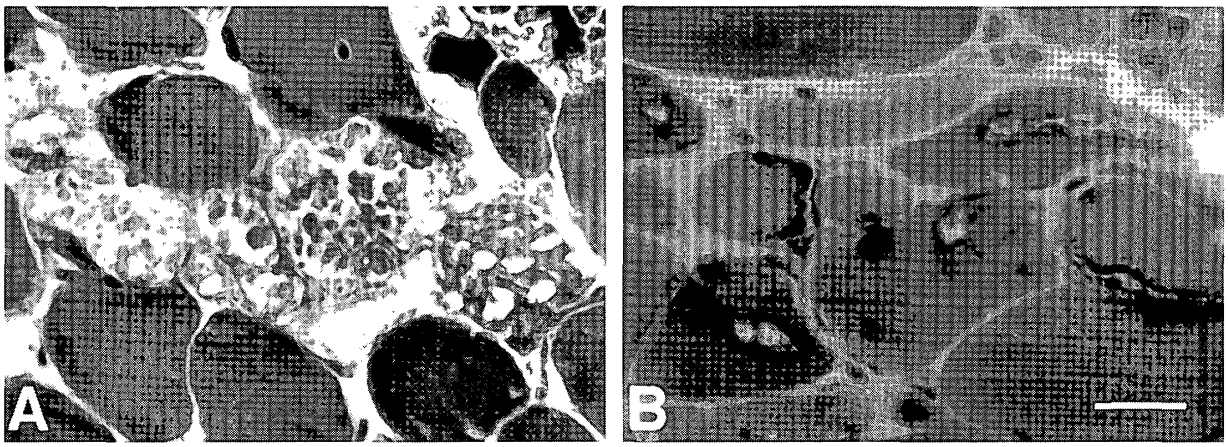

Fig. 3. Histochemistry. Transverse serial sections of muscle of patient with acid maltase deficiency (A) and DMRV/HIBM (B). A: Intracytoplasmic vacuoles are so large that they occupy most of the space in many muscle fibers. These vacuoles contain amorphous materials, strongly highlighted with periodic and Schiff staining, indicating that contain glycogen. B: There are many fibers with vacuoles surrounded or rimmed by granular material (rimmed vacuoles). Modified Gomori trichrome stain. Bar 30 μm.

More than 50 mutations have been revealed in the gene encoding acid maltase (acid α-glucosidase), which is located on chromosome 17q25.[11] Generally, null and deletion-type mutations cause virtually complete loss of enzyme activity and are associated with infantile AMD phenotype.

In muscle pathology, the number and the size of vacuoles in the muscle fiber are largely proportional to clinical severity. Many large vacuoles are seen in the infantile form (Fig. 3), but they are not as prominent in the adult-onset form. In the infantile form, intracytoplasmic vacuoles are so large that they occupy most of the space in many muscle fibers. These vacuoles contain amorphous materials with H&E or modified Gomori trichrome stains, and have high acid phosphatase activity, reflecting the lysosomal nature of the vacuoles. They are also strongly highlighted with periodic and Schiff staining, indicating that they contain glycogen. Electron microscopy shows that vacuoles contain cytoplasmic debris, electron dense bodies, and myelin figure, in addition to glycogen particles. Glycogen deposition is usually more prominent outside the vacuoles.

2. AUTOPHAGIC VACUOLAR MYOPATHIES WITH SARCOLEMMAL FEATURE AND ACETYLCHOLINESTERASE ACTIVITY

Another category of autophagic vacuolar myopathies (AVM) includes Danon disease[8,12] and X-linked vacuolar myopathy with excessive autophagy (XMEA).[13] These myopathies share two unique pathologic findings: 1) structural features of sarcolemma and 2) biochemical activities of acetylcholinesterase (AChE) and non-specific esterase in the autophagic vacuolar membranes.[14,15] Most sarcolemmal proteins including dystrophin have been detected in vacuolar membranes. This unique intracytoplasmic membrane structure was not found in other categories of AVM including AMD and rimmed vacuolar myopathies. In addition to these two well-characterized diseases, there are likely to be more myopathies in this category, including infantile AVM[16], AVM with late-onset and multiorgan

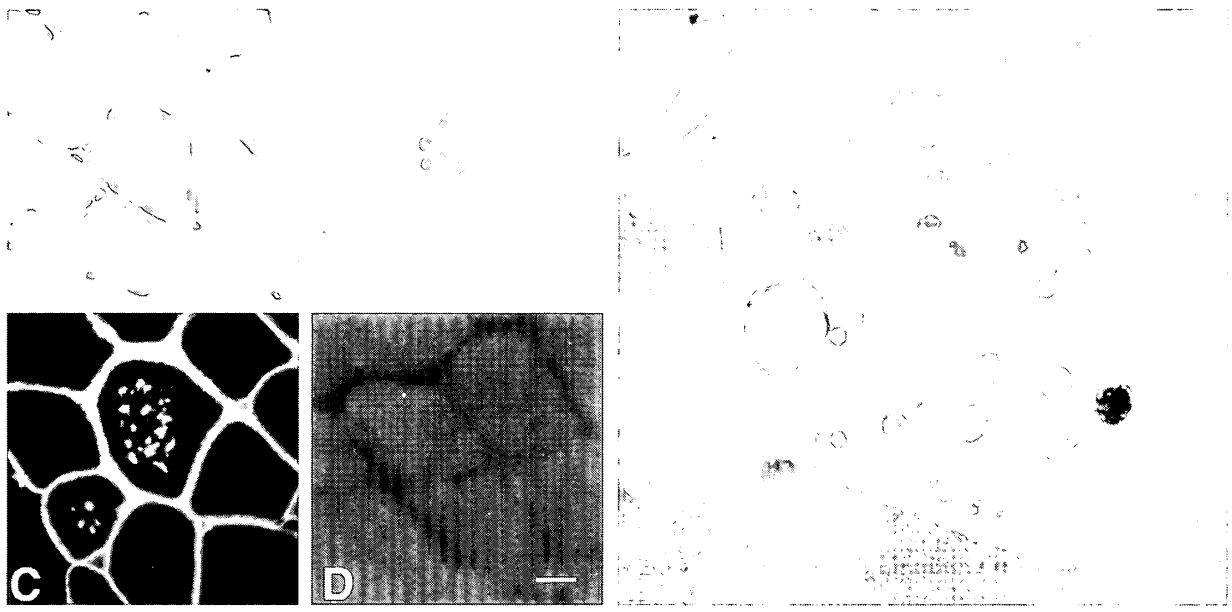

Fig. 4. Histochemistry and immunohistochemistry. Transverse sections of skeletal muscle biopsies from Danon disease patient. Several fibers contain scattered tiny basophilic intracytoplasmic vacuoles (A: H&E stain). The vacuolar membrane has high acetylcholinesterase activities (B). Sections were immunostained with antibody against dystrophin (C: FITC-labeled staining). LAMP-2 (D) is completely absent in muscle. Electron microscopy revealed that the intracytoplasmic vacuoles typically contain myelin figures, electron-dense bodies, and various cytoplasmic debris. Basal lamina is seen along the inner surface of autophagic vacuoles (E). A-D: Bar 25 μm. E: Original magnification: ×15,000.

involvement[17], and others.

Nevertheless, these diseases are likely to share a common pathomechanism because of very unique pathologic features of autophagic vacuoles. Although the pathomechanism underlying this unique pathologic phenomenon is a mystery, these AVM are likely to have genetic defects in a common pathway important for lysosomal function.

Danon Disease

Danon disease was originally described as "lysosomal glycogen storage disease with normal acid maltase" by Danon et al. in 1981 because the patients had a disease clinicopathologically similar to acid maltase deficiency but had normal enzymatic activity.[18] However, the disease is not a glycogen storage disease because glycogen is not always increased and because the primary defect resides in lysosome-associated membrane protein-2 (LAMP-2), a lysosomal structural protein rather than a glycolytic enzyme.

Clinically, Danon disease is characterized by the triad of hypertrophic cardiomyopathy, myopathy, and mental retardation.[12,19] All probands have been male, but female patients do develop a milder, later-onset cardiomyopathy; therefore, the disease is transmitted in X-linked dominant mode of inheritance. In fact, the causative gene for Danon disease, *LAMP-2*, is present on chromosome Xq24.[8] Moreover, in female patients, we revealed a 50% reduction of LAMP-2 expression ("LAMP-2 haploinsufficiency") on immunohistochemistry and western blot analyses.[20] All patients develop cardiomyopathy, which is the most severe and life-threatening manifestation. In male patients, hypertrophic cardiomyopathy and

cardiac arrhythmia are common clinical signs. Skeletal myopathy is usually mild and is evident in most male patients, but is present in only one third of female patients. Mental retardation is usually mild and is present in 70% of male patients, but is absent in most female patients.

Muscle pathology demonstrates many scattered intracytoplasmic vacuoles, which on H&E stain often look like tiny basophilic granules (Fig.4). Usually, no necrotic or regenerating fibers are seen. The membranes of these vacuoles have structural features of sarcolemma. Actually, we revealed that the autophagic vacuolar membranes in Danon disease have most of the sarcolemmal proteins ranging from cytoplasmic dystrophin to extracellular laminin. In addition, the vacuolar membranes have biochemical activities of AChE and non-specific esterase.[12,15] However, because the membranes lacked acetylcholine receptors (AChRs), the presence of AChE without AChRs clearly indicates that the vacuolar membranes are distinct from either junctional or extra-junctional sarcolemma and suggests that they are formed through a unique process. Electron microscopy revealed that the intracytoplasmic vacuoles typically contain myelin figures, electron-dense bodies, and various cytoplasmic debris; therefore, they are autophagic vacuoles. Interestingly, basal lamina is sometimes seen along the inner surface of autophagic vacuoles, providing further evidence that the vacuolar membrane has features of sarcolemma.

The autophagic nature of the vacuoles on electron microscopy indicate that most autophagic vacuoles are autolysosomes.[15] Small basophilic granules on H&E stain are most likely these autolysosomal accumulations as suggested by their pattern of distribution and the fact that lysosomes are basophilic on H&E stain. In some, but not all fibers, these autolysosomal accumulations were surrounded by membranes with dystrophin reactivity by immunohistochemistry. These findings indicate that some clusters of autolysosomes are surrounded by membranes with sarcolemmal features but others are not. In support of this notion, ultrastructural studies identified two types of autophagic vacuoles: 1) clusters of autophagic vacuoles, not surrounded by membranes or basal lamina, and 2) vacuoles containing various autophagic material encircled by membranes with basal lamina along the luminal side.

More recently, we have shown that the number of fibers with autophagic vacuoles surrounded by membranes increased linearly with age while the number with autolysosomal accumulations decreased slightly in Danon disease. These findings have suggested that autophagic vacuoles are produced secondarily in response to autolysosomes and that most autophagic vacuoles form enclosed spaces, indicating that the vacuolar membranes may be formed in situ rather than through sarcolemmal indentation.[21]

X-Linked Myopathy with Excessive Autophagy

X-linked myopathy with excessive autophagy (XMEA) was reported in 1988 by Kalimo et al as a new type of AVM in a Finnish family.[13] The disease is transmitted in an X-linked recessive manner. Clinically, this is a childhood-onset slowly progressive disease of skeletal muscle with no cardiac, nervous system, or other organ involvement. Muscle pathology shows many tiny vacuoles, and the vacuolar membranes have features of plasma membrane as in Danon disease. Autophagic vacuoles are seen in the cytoplasm. The muscle biopsy

Table 2. Summary of autophagic vacuolar myopathies (AVM)

Disease	Inheritance	Locus	Gene product
1. Primary lysosomal myopathies			
1. Acid maltase deficiency	AR	17q25	Acid maltase
2. AVM with sarcolemmal features and AChE activity			
1) Danon disease	XD	Xq24	LAMP-2
2) X-linked myopathy with excessive autophagy	XR	Xq28?	?
3) Infantile AVM	AR or Sp	?	?
4) AVM with late-onset and multiorgan involvement	AR or Sp	?	?
5) Others			
2. Secondary lysosomal myopathies			
1. Hereditary rimmed vacuolar myopathies			
1) DMRV/HIBM	AR	9p1-q1	GNE
2) Limb-girdle muscular dystrophy 2G	AR	17q12	Telethonin
3) Tibial muscular dystrophy	AD	2q24.3	Connectin/Titin
4) Desmin myopathy	AD	2q35	Desmin
5) Oculopharyngeal muscular dystrophy	AD	14q11.2	PABP2
6) Limb-girdle muscular dystrophy 1A	AD	5q31	Myotilin
2. Non-hereditary rimmed vacuolar myopathies			
1) Sporadic inclusion body myositis			
2) Others			

AR; autosomal recessive, XD; X-linked dominant, XR; X-linked recessive, AD; autosomal dominat, Sp; Sporadic, AChE; Acetylcholinesterase, LAMP-2; lysosome-associated membrane protein-2, DMRV; distal myopathy with rimmed vacuoles, HIBM; hereditary inclusion body myopathy, PABP2; poly (A) binding protein 2, GNE; UDP-GlcNAc 2-epimerase/Man NAc kinase.

resembles that of Danon disease; therefore, the two diseases are likely to share similar molecular pathomechanisms. The characteristic pathology in XMEA is intense deposition of membrane attack complex (complement C5b-9) at the myofiber surface and multifold reduplication of the basement membrane, which are not seen in Danon disease. Moreover, the presence of LAMP-2 in XMEA muscle clearly shows that XMEA is distinct from Danon disease. In addition, XMEA has been linked to the most telomeric 10.5 cM of Xq28,[22] whereas the gene encoding LAMP-2 is present on Xq24.

Infantile Autophagic Vacuolar Myopathy

Two well-documented infants with AVM were reported as having the infantile form of "lysosomal glycogen storage disease with normal acid maltase".[16] Both patients presented with muscle weakness and hypotonia at birth and died early in their lives. Muscle pathology demonstrated extensive vacuolar changes with increased glycogen, but acid maltase activity was normal in both patients; therefore, the diagnosis seemed to be clear. Nevertheless, the infantile disease is distinct from Danon disease because LAMP-2 protein is not deficient in the skeletal muscle and sequences of the *LAMP-2* gene are normal. Interestingly, as in XMEA muscle, membrane attack complex (C5b-9) stained muscle sarcolemma in this disease. Electron microscopy showed that many vacuoles containing membrane-bounded glycogen particles, free glycogen particles, and cytoplasmic degradation products scattered in the

cytoplasm. Moreover, duplication of basal lamina into two layers was observed along portions of the sarcolemma. Multilayered basal lamina was seen in some fibers. Material exocytosed from vacuoles accumulated under and between the multiple layers of basal lamina. The deposition of membrane attack complex over the surface of muscle fibers and the multiplication of basal lamina suggest that the pathological features of infantile AVM are more similar to those of XMEA than those of Danon disease.

Other Autophagic Vacuolar Myopathy

Autophagic vacuolar myopathy with late-onset and multiorgan involvement was reported as a novel form of adult-onset AVM.[17] This disease involves multiple organ systems including eyes, heart, liver, lung, kidney, and skeletal muscle. The vacuolar membranes had sarcolemmal features similar to vacuoles in Danon disease, XMEA, and infantile AVM. LAMP-2, absent in Danon disease, was present. Defined by distinct clinical features, this disease constitutes the fourth entity in this category of AVM in which the vacuolar membranes have features of sarcolemma.

Furthermore, recently, another novel form of AVM has been reported, entitled "A novel form of infantile AVM".[23,24] This disease shows an autosomal recessive inheritance pattern. Pathologically, the vacuolar membranes had sarcolemmal features and AChE activity similar to vacuoles in Danon disease and related myopathies. LAMP-2 was present. From now on, most likely there will be other diseases discovered in this category.

3. RIMMED VACUOLAR MYOPATHIES

Rimmed vacuoles are one of the most frequently encountered lysosomal abnormalities in muscle pathology.[7,25] Rimmed vacuoles are typically detected as small vacuoles lined by many red granules (the "rim") on modified Gomori trichrome staining (Fig.3). However, these vacuoles are not true holes in the muscle, but rather artifacts produced during the staining procedure. Rimmed vacuoles are clusters of autophagic vacuoles and myeloid bodies ultrastucturally. These autophagic vacuoles probably detach easily from glass slides and move to the nearby myofibrils during the staining procedure. The regions where autophagic vacuoles had been clustered become empty (vacuoles), and the surrounding areas are decorated by granular autophagic vacuoles (rim).

There are a number of not only non-hereditary but also hereditary muscular disorders pathologically characterized by the presence of rimmed vacuoles. Interestingly, none of the genes responsible for the diseases in this category encode lysosomal proteins; they all encode extralysosomal proteins. Therefore, rimmed vacuoles are thought to be formed by not primary but secondary abnormalities of lysosome, and we consider rimmed vacuolar myopathies as 'secondary lysosomal myopathies'.

Distal Myopathy with Rimmed Vacuoles / Hereditary Inclusion Body Myopathy

Nonaka et al reported originally in 1981 distal myopathy with rimmed vacuoles (DMRV) as a new type of distal myopathy.[25] This disease is characterized by preferential involvement of tibialis anterior muscle and rimmed vacuoles in muscle pathology (Fig.3). Argov et al reported in 1984 a disease similar to DMRV under the name of rimmed vacuole myopathy

sparing the quadriceps.[26] The latter disease is now widely known as hereditary inclusion body myopathy (HIBM). Recently, HIBM was shown to be associated with mutations in the gene of UDP-GlcNAc 2-epimerase/Man NAc kinase (*GNE*).[27] More recently, DMRV was also shown to be associated with *GNE* gene mutations.[28] Therefore, DMRV and HIBM are allelic and most likely are the same disease.

The *GNE* gene encodes a single protein with bifunctional enzyme activities. The protein catalyzes the rate-limiting step in the sialic acid biosynthetic pathway. The epimerase activity is reduced in lymphocytes from DMRV patients regardless of the site of the mutation, indicating that DMRV is associated with the loss of function of the GNE enzyme. Therefore, the primary molecular defect resides outside of the lysosomes, indicating that rimmed vacuoles are secondarily activated lysosomes and autophagic vacuoles.

Sporadic Inclusion Body Myositis

Sporadic inclusion body myositis (SIBM) is a chronic inflammatory, corticosteroid-resistant myopathy with weakness of both proximal and distal muscles.[29] In patients suspected of SIBM, polymyositis (PM) is part of the differential diagnosis. As PM is responsive to immunosuppressive treatment and SIBM generally is not, it is important to differentiate SIBM from PM in an early stage. Although both diseases differ in age of onset, distribution of weakness and rate of progression, the definite diagnosis of SIBM rests on the muscle biopsy. SIBM is differentiated from PM by the presence of basophilic rimmed vacuoles in non-necrotic muscle fibers in the H&E stain, whereas endomysial lymphocytic infiltrates are indistinguishable in both diseases.

Recently, it has been shown that the over-expression of β amyloid peptide precursor (β-APP) induced inclusion body myositis-like phenotype both in vitro[30] and in vivo.[31] The autophagic process is probably secondarily activated to degrade them, although lysosomal abnormalities have not been specifically documented. In support of this notion, other degradation systems, such as the ubiquitin-proteasome system and even apoptotic system, are also commonly activated in many of the diseases in this category, indicating that lysosomal abnormality is not the primary phenomenon. Therefore, all rimmed vacuolar myopathies are plausibly secondary lysosomal myopathies.

CONCLUSIONS

More recently, it has been understood that autophagy plays an important role not only in health but also in diseases. Among various diseases associated with autophagy, there are autophagic vacuolar myopathies. After the discovery of the causative gene of Danon disease, the concept of autophagic vacuolar myopathies has been understood. In autophagic vacuolar myopathies except rimmed vacuolar myopathies, the primary defects are likely to reside in lysosomes, as has been shown in Danon disease and acid maltase deficiency. Further, the most characteristic features of autophagic vacuolar myopathies including Danon disease are that autophagic vacuolar membranes have the feature of sarcolemma and AChE activity. These unique findings are very unusual and are never found in other autophagic vacuolar myopathies. Although the concept of autophagic vacuolar myopathy or lysosomal myopathy is not yet well established, most likely there will be other diseases discovered in this

category. In addition, further studies can be expected to lead to the elucidation of the mechanism of these peculiar vacuolar membranes and the therapeutic strategy for lysosomal defects.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Ichizo Nishino and Dr. Ikuya Nonaka (National Center of Neurology and Psychiatry, Japan) for their kind suggestions and advice on this work.

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