Pexophagy is responsible for 65% of cases of peroxisome biogenesis disorders

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ABSTRACT
Peroxisome biogenesis disorders (PBDs) is a group of diseases caused by mutations in one of the peroxins, proteins responsible for biogenesis of the peroxisomes. In recent years, it became clear that many peroxins (e.g., PEX3 and PEX14) play additional roles in peroxisome homeostasis (such as promoting autophagic degradation of peroxisomes or pexophagy), which are often opposite to their originally established functions in peroxisome formation and maintenance. Even more interesting, the peroxins that make up the peroxisomal AAA ATPase complex (AAA-complex) in yeast (Pex1, Pex6 and Pex15) or mammals (PEX1, PEX6, PEX26) are responsible for the downregulation of pexophagy. Moreover, this might be even their primary role in human: to prevent pexophagy by removing from the peroxisomal membrane the ubiquitinated peroxisomal matrix protein import receptor, Ub-PEX5, which is also a signal for the Ub-binding pexophagy receptor, NBR1. Remarkably, the peroxisomes rescued from pexophagy by autophagic inhibitors in PEX1G843D (the most common PBD mutation) cells are able to import matrix proteins and improve their biochemical function suggesting that the AAA-complex per se is not essential for the protein import function in human. This paradigm-shifting discovery published in the current issue of Autophagy has raised hope for up to 65% of all PBD patients with various deficiencies in the AAA-complex. Recognizing PEX1, PEX6 and PEX26 as pexophagy suppressors will allow treating these patients with a new range of tools designed to target mammalian pexophagy.

Peroxisomes are the organelles that carry out many important functions in human metabolism. As a consequence, inherited mutations in many of the peroxisomal genes can lead to peroxisomal disorders. Most of the peroxisomal disorders can be grouped into either single peroxisomal enzyme deficiencies (PEDs) or peroxisome biogenesis disorders (PBDs) characterized by multiple abnormalities due to a defect in the formation or maintenance of peroxisomes. The latter 2 functions are supported by the peroxisome biogenesis factors or peroxins encoded by the PEX genes. The bi-allelic recessive mutations in 14 PEX genes, including PEX1, PEX6 and PEX26 that encode the subunits of the peroxisomal AAA ATPase complex (AAA-complex), have been reported to cause most of the PBDs in human (for a review, see ref. 1). Interestingly, mutations in the AAA-complex genes, PEX1 (48.5%), PEX6 (13.1%) and PEX26 (3.4%), are the most common among PBD patients and account for 65% of all the PBD cases.2

Until recently, it was widely accepted that the AAA-complex participates mainly in the import of the peroxisomal matrix proteins, because the cells from corresponding PBD patients have membrane remnants of the peroxisomes with properly localized peroxisomal membrane proteins. Precisely, the heteromeric complex of PEX1 and PEX6 ATPases recruited to the peroxisomal membrane by PEX26 (Pex15 in yeast or APEM9 in plants) drives the ATP- and ubiquitin-dependent release of the ubiquitinated peroxisomal matrix protein import receptor, Ub-PEX5, from the peroxisomal membrane to the cytosol. Such dislocation of Ub-PEX5 from peroxisomes and its deubiquitination (mediated by Pex6-bound Ubp15 in yeast) are required for receptor recycling and repeated rounds of import of the peroxisomal matrix proteins that contain the peroxisomal targeting signal 1 (PTS1). It was also proposed that the ATPase-mediated removal of PEX5 from the peroxisomes might serve as a pulling force for PTS1-cargo translocation across the peroxisomal membrane (i.e., export driven import). For reviews on these topics, see refs. 3, 4

Before the peroxisomal matrix protein import function of the AAA-complex dominated the peroxisome field, a beautiful set of studies in yeast suggested that Pex1, Pex6 and ATP hydrolysis were required for the heterotypic fusion (at priming and docking steps) of small peroxisomal vesicles, P1 and P2, which are the earliest precursors of mature peroxisomes.3,6 Such a fusion was later proposed to be required for the assembly of a complete peroxisomal translocon, which imports matrix proteins, since each of the 2 peroxisomal vesicles carried only half of the peroxins involved in peroxisomal matrix protein import.7 However, subsequent studies did not confirm the existence of half-translocons in the peroxisomal membrane remnants of pex1 and pex6 cells. Instead, these remnants contained a complete translocon but no matrix proteins supporting an essential role of Pex1 and Pex6 in peroxisomal matrix protein import.8,9 The 2 established functions of Pex1 and Pex6 in
(1) fusion of the pre-peroxisomal vesicles and (2) export of the ubiquitinated Pex5 from the peroxisomal membrane (for peroxisomal matrix protein import) are analogous to the roles of 2 other AAA ATPases, NSF (Sec18 in yeast)—in membrane fusion, and VCP/p97 (Cdc48 in yeast)—in export of the ubiquitinated proteins from the ER membrane. The homohexameric complexes of NSF and VCP have distinct structural features that might help to model the molecular function(s) of the heterohexameric Pex1-Pex6 complex. However, despite the fact that the first structures of the Pex1-Pex6 complex were solved recently, an accurate prediction of its molecular role(s) in the cell must await further studies (for a review, see ref. 10).

Recently, the yeast AAA-complex was implicated in the 3rd function: prevention of pexophagy.11 Pexophagy is the selective autophagic degradation of peroxisomes. This process is necessary for removal (from the cytosol) and recycling (in the lysosomes or vacuoles) of superfluous and/or damaged peroxisomes (for a review, see ref. 12). Interestingly, lack of Pex1, Pex6 or Pex15 triggers degradation of the peroxisomal membrane remnants that depends on the yeast pexophagy receptor, Atg36. It is remarkable that depletion of Pex1 in yeast first causes the peroxisomal matrix protein import defect before it results in pexophagy. However, the buildup of Ub-Pex5 in the peroxisomal membrane is not a trigger for pexophagy in yeast.11 In this issue of *Autophagy*, Peter Kim and colleagues report that in human cells the AAA-complex also prevents pexophagy mediated (in this case) by the Ub-binding pexophagy receptor, NBR1.13 In contrast to yeast pexophagy, this process in the AAA-complex-depleted human cells depends on the accumulation of Ub-Pex5 in the peroxisomal membrane. In this regard, the role of this protein is consistent with 2 other recent reports that identified mammalian Ub-PEX5 as an important pexophagic signal.14,15

Strikingly, the loss of a functional AAA-complex in human cells does not per se abrogate peroxisome formation or peroxisomal matrix protein import—rather, the enhanced pexophagy does. Law et al. have elegantly shown that if you titrate the excessive pexophagy with autophagic inhibitors, such as chloroquine, the fibroblasts of a patient with the most common PBD mutation, PEX1G843D (that causes a mild form of PBD), will not only restore their peroxisome number but also PTS1-protein import and peroxisomal β-oxidation of very long-chain fatty acids.13,15 Apparently, the recycling of Ub-PEX5 is not essential for peroxisomal matrix protein import in human. The export of Ub-PEX5 from the peroxisomal membrane via the AAA-complex is more important for the prevention of pexophagy than the recycling of PEX5 and PTS1-protein import (Fig. 1). Indeed, in contrast to yeast, the mammalian cells use Ub as a degradation signal for many selective autophagy pathways, including pexophagy.16 Therefore, a continuous removal of the ubiquitin and/or ubiquitinated proteins from the intracellular organelles, such as peroxisomes, must be an essential housekeeping mechanism. In addition, if human (but not yeast) cells have an excess of cytosolic PEX5, it could explain why the PEX5 recycling function of the AAA-complex is secondary in human.

Next, it will be interesting to see how this pexophagy prevention function of the human AAA-complex is terminated under pexophagy-inducing conditions. A possibility exists that the AAA-complex or its single component is degraded ahead of the peroxisomes when pexophagy is induced. Such degradative inactivation of the AAA-complex would allow Ub-PEX5 to recruit NBR1 to the peroxisomal membrane and trigger pexophagy. Recently, it was demonstrated that both PEX1 and PEX6 contain evolutionarily-conserved Atg8-interacting motifs.17 Moreover, for plant PEX6, the interaction with Atg8 was also shown experimentally, suggesting that at least PEX6 (if not both peroxisomal ATPases) might indeed be a substrate of the selective autophagic degradation that contributes to the induction of pexophagy from yeast to human.

Currently, there is no curative therapy for the PBDs (for reviews of symptomatic therapy, see refs. 18, 19). Recognition of PEX1, PEX6 and PEX26 as pexophagy suppressors opens up an exciting opportunity to cure the majority of PBD patients. The Kim group has already reported that daily addition of a small, nontoxic amount of chloroquine (5 μM) in the growth medium to suppress autophagic pathways supported PTS1-protein import in PEX1G843D fibroblasts for up to 15 d in cell culture.13 This result suggests that targeting of the human pexophagy-specific proteins could potentially provide even better therapeutic outcomes (stronger inhibition of pexophagy leading to even better peroxisomal matrix protein import, combined with intactness of other autophagic pathways enabling even greater cell survival). However, such an ideal target would also have to be dispensable for peroxisome biogenesis and metabolism. Unfortunately, a protein satisfying all of these criteria is unknown yet.

Interestingly, the effects described by Law et al. for chloroquine (increased peroxisome number, matrix protein import
and biochemical function) in PEX1G843D cells were previously reported for: (1) nonspecific chemical chaperones, such as 4-phenoxybutyrate (4-PBA), trimethylamine N-oxide (TMAO), glycerol and arginine, and (2) PEX1G843D-specific chaperones, such as acacetin diacetate (AD), aminoalkyl bisindolylmaleimide (GF109203x) and structurally related Ro 31–8220. Despite the fact that other mechanisms were proposed to explain the pharmacological effects of these molecules (e.g., increased transcription of ABCD2/ALDR and PEX11A/PEX11α genes in response to 4-PBA or competitive and reversible binding of AD, GF109203x and Ro 31–8220 to the ATP binding sites on PEX1G843D), many of them also modulate autophagy. For example, 4-PBA inhibits ER stress-induced autophagy, attenuates IFNG (interferon gamma)-induced autophagy, and AD, GF109203x and Ro 31–8220 might all inhibit protein kinase C required for pexophagy signaling in yeast and, most probably, in mammalian cells (at least under hypoxic conditions). Therefore, it is possible that previous successes in the pharmacological induction of peroxisomes in patient fibroblasts with mild AAA-complex deficiencies were also due to attenuation of pexophagy.

In the future, it will be important to examine the effectiveness of chloroquine treatment in restoring the AAA-complex defects in vivo. Luckily, the murine model of human PEX1G843D deficiency, the PEX1G844D mouse, has been developed recently. This model recapitulates many features of the human disorder, including the responsiveness of murine PEX1G844D cells to chemical chaperones. Therefore, Kim and colleagues are poised to test if their paradigm-shifting discovery (that a primary role of the human AAA-complex is to prevent pexophagy) can change the way we treat up to 65% of PBD patients who display excessive pexophagy.

Disclosure of potential conflicts of interest

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