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EDITORIAL

Autophagy in neuropathology

Berge A. Minassian · Hannu Kalimo

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Christian de Duve discovered lysosomes [8] by "Exploring Cells with a Centrifuge" as was the title of his Nobel lecture when he in 1974 received the Nobel prize in Physiology and Medicine. He and his team found that acid phosphatase activity in liver homogenates was latent because it was enwrapped in "membrane sacs", membrane-bound organelles, whereby substrates were made inaccessible to this and other hydrolytic enzymes with various specificities and optimal activities at acidic pH. The biochemical discovery was translated into morphology above all by the pioneering electron microscopic studies by Novikoff et al. [14] and Clark [5]. According to de Duve [10] it was Novikoff and Essner [15] and Ashford and Porter [1] who discovered autophagy having made the key morphological observations. A most important meeting in the lysosome–autophagy research was the Ciba Foundation Symposium on Lysosomes in 1963 [4, 10], where de Duve presented a paper "The lysosome concept" and Novikoff presented his characterization of cytolysomes, acid phosphatase-positive organelles—thus, clearly related to lysosomes—which contained various cytoplasmic components, such as mitochondria, ER membranes and ribosomes in an obvious state of disintegration. It was at that important meeting, where de Duve suggested the terms endocytosis and exocytosis as well as distinguished heterophagic from autophagic functions of lysosomes, and he succeeded in persuading Novikoff to replace the name cytolysomes with the term autophagic vacuoles. Early simple hypotheses were that lysosomes are "suicide bags" the rupture of which release lytic enzymes leading to physiological autolysis [10] and autophagy serves as a cellular rubbish-disposal mechanism [22]. The highly productive research after the discoveries above has shown that this 'self-eating' process not only participates in the disposal of intracellular misfolded or "outdated" long-lived proteins, unnecessary/excessive or damaged organelles, but also is a supportive response to provide nutrients and energy to cells exposed to various stresses, such as starvation. The history of the rapidly accelerating progress in autophagy research has been reported for example by Yang and Klionsky [22] and Ohsumi [17], all leading figures in this field. From the 1960s to early 1980s, autophagy studies relied on enzyme bio- and histochemistry as well as light and electron microscopic morphology. The "tsunami" of molecular biology and genetics swept over autophagy in the 1990s, when the molecular basis of autophagy began to emerge. This progress has set tough methodological demands to autophagy researchers, as described in the united guideline article by an extensive (well over 1,000 authors) squad of renowned and influential autophagy researchers for the use and interpretation of assays for monitoring autophagy [11].

The importance of autophagy in cellular homeostasis is reflected in the vast number of recently published scientific articles, a great share of them dealing with autophagy in neuropathology, i.e. its role in the health and disease of the nervous and muscle tissues. The importance of autophagy in the homeostasis and survival of these two tissues reflects the fact that neurons and mature myofibers are postmitotic cells and

B. A. Minassian
Program in Genetics and Genome Biology and Division of Neurology, Department of Paediatrics, The Hospital for Sick Children, Toronto, ON, Canada

H. Kalimo (✉)
Department of Forensic Medicine, Institute of Biomedicine, University of Turku, Kiinamyllynkatu 10, 20520 Turku, Finland
e-mail: hannu.kalimo@helsinki.fi

H. Kalimo
Department of Pathology, University of Helsinki, Helsinki, Finland

therefore they cannot dilute the deleterious burden of toxic substances and dysfunctional organelles by cell division [7]. This vulnerability of nervous tissue is manifest in neurodegenerative diseases, in which accumulation of aggregates of many different types of misfolded or excessive proteins occurs in increasing amounts with ageing (e.g. [7, 16]).

As for skeletal muscle/mature myofibers, they not only move us, but are also one of the most important sites to control body metabolism [19]. Therein autophagy has an important physiological role in maintaining the homeostasis of skeletal muscle. For example, autophagy is needed for exercise-induced improvement of physical performance [12]. Autophagy does not only contribute to anabolic consequences of training, but is also pivotal in clearing proteins and organelles damaged mechanically (contractions and injuries) and/or metabolically (e.g. by reactive oxygen species produced by active mitochondrial energy production). Furthermore, autophagy also has a major role in catabolic conditions, both muscle specific such as denervation, inactivity and different myopathies and muscular dystrophies and systemic such as fasting, cancer, diabetes, sepsis and AIDS [13, 20, 21].

This cluster includes four review articles on autophagy and its role in selected neuropathological entities. The large group of lysosomal storage diseases (LSD), more than 50 classic LSDs, majority of which arise from monogenic defects in degradative pathways, and 14 variable neuronal ceroid lipofuscinoses, which are being included among LSDs (e.g. [3]), are beyond the scope of this limited cluster.

Firstly, the review by Damme together with Suntio, Saftig and Eskelinen describes both autophagy in general and the specific aspects of autophagy in neuronal cells, in which—as mentioned also above—"autophagy is especially important for the survival and homeostasis of postmitotic cells like neurons, because these cells are not able to dilute accumulating detrimental substances and damaged organelles by cell division". The authors also point out that non-renewable neurons need autophagy as a non-cytolytic mechanism to combat viral infections.

The role of key molecules is described both in words and in vivid and highly informative illustrations. They also present the importance and activity of trafficking within neurons and their long processes as well as the roles of basal vs. induced and selective vs. non-selective autophagy. Effects of drug-induced upregulation or inhibition of autophagy as well as effects of either defective or excessive autophagy in mice with central nervous system or neuronal cell-type-specific knock-out of autophagy proteins are also discussed. The role of autophagy both at the beginning of life in neurogenesis and towards the end of life in ageing, as well as in a selected group of neurodegenerative diseases, amyotrophic lateral sclerosis, fronto-temporal dementia, various lysosomal storage disorders, Parkinson´s disease and prionopathies is described. In this review, the close association of autophagy with the other, proteasomal, degradation pathway of misfolded and "undesired" proteins is also touched upon [2, 18].

In their review, Peric and Annaert take a novel interesting approach to the role of autophagy in Alzheimer´s disease (AD). It is not surprising that dysfunctional autophagy is somehow associated with the pathological deposition of aggregation-prone amyloid β peptide in extracellular amyloid plaques and hyperphosphorylated tau in intraneuronal neurofibrillary tangles (NFTs) and lead to synaptic abnormalities and neuronal degeneration in AD, although the exact pathogenic contribution of these two AD hallmarks is not fully clarified. In this review, the authors summarize the pathogenic events, abnormalities in the endosomal sorting–lysosomal trafficking and autophagic degradation, which may precede and lead to the two hallmark alterations of AD. They furthermore discuss how these dysfunctions may influence neurodegenerative processes, not only in AD but also in other neurodegenerative diseases, for example fronto-temporal dementia, Parkinson's disease and polyglutamine tract diseases.

The two reviews by Dowling et al. and Endo et al. deal with myopathies with similar morphological findings, but with pathogenetically different though quite possibly related defects in autophagy functions. In X-linked myopathy with excessive autophagy (XMEA), the defect lies in impaired acidification of autolysosomes due to insufficiency of the vacuolar ATPase proton pump, with consequent block in the degradation of the cargo within autolysosomes by lysosomal acidic hydrolases. The vacuolar ATPase deficiency results from hypomorphic mutations in the *VMA21* gene, the main endoplasmic reticulum chaperone involved in assembling this multimeric proton pump. In Danon disease, the defect is in the LAMP-2B isoform of the lysosome-associated membrane protein-2. Numerous functions have been attributed to this protein, but one that is best documented is its role in autophagosome maturation, i.e. fusion of autophagosomes with lysosomes and consequent acquisition of vacuolar ATPases, acid (protons) and acidic hydrolases. In both diseases, therefore, failure of macroautophagy appears to be at least part of the underlying pathophysiology. In both cases, the prominent pathological feature is the presence of large vacuoles containing partially or incompletely degraded cellular components, such as disintegrated mitochondria, various membranes, and glycogen. Studies in XMEA suggest that the failure of macroautophagy induces feedback upregulation of autophagosome formation, and the proliferating autophagosomes, unable to complete their autophagic cycles, fuse one with another to generate the large vacuoles. XMEA and Danon disease, however, are not identical disorders, the former restricted wholly to skeletal muscle, the latter involving maximally the heart, in the

former the vacuoles travelling to the sarcolemma and exocytosing their contents, in the latter not. Much more needs to be understood about these two diseases, but both certainly are diseases of failed macroautophagy. In both cases, the failed autophagy is in the final degradative stage, not the initial stages of autophagy. In fact, as mentioned, the unaffected initial steps of autophagy may be part and parcel of the problem, namely in their hyperactivation in response to the failing completion of the process in its final degradative stage. In fact, other diseases that also block the final stage of autophagy lead to similar vacuolation. One is chloroquine myopathy, an iatrogenic disease in which this antimalarial agent alkalinizes lysosomes and thus hinders the final stage of autophagy. Another is a recently uncovered allelic variant of Batten's disease, ceroid lipofuscinosis type 3 (CLN3) [6, 9]. The deficient protein (battenin) in CLN3 has long been known to play a role in lysosomal acidification, and a well-known diagnostic test in this disease is the identification of vacuolated lymphocytes in peripheral blood. Most cases with Batten's disease have such an aggressive and rapid brain demise that it is not known whether meanwhile, or later, other organs might also have been affected. Recently, a CLN3 mutation was described in which the brain disease was extremely mild and slowly progressive [6]. The affected brothers presented with a vacuolar myopathy closely resembling XMEA or Danon disease during their unusually prolonged course taking them into their forties, suggesting that Batten's disease might "contain" a vacuolar myopathy, left subclinical in the short span of most patients' lives. Why do Batten's disease patients suffer a horrendous neurodegenerative disease of the brain, while Danon patients have inconsistent and mild cognitive difficulties, and XMEA patients no brain disease at all is an area rife for the understanding of the particularities of autophagy in various organs, including the brain. Finally, if all these diseases are indeed, even if in part, due to partial alkalinization of autophagic machinery, their therapies might be relatively easily attainable through identification of means of partial correction of the acid in de Duve's organelle and its frequent partner, the autophagosome.

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