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VERSITA

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AUTOPHAGOSOMES AND MULTIVESICULAR BODIES IN NEURONAL DEVELOPMENT AND DEGENERATION

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Abstract

A growing body of research deals with the relationship between the endosomal and autophagic/lysosomal pathways during developmental stages of the central nervous system. This includes their possible influence regarding the onset and progression of specific neurodegenerative disorders. In this review we focus our attention on major alterations affecting two organelles: autophagosomes and multivesicular bodies, both of which are located at the intersection point of their respective pathways.

Keywords

• Autophagosomes • Multivesicular bodies • Neuronal development • Neurodegeneration

Autophagosomes and multivesicular bodies: two actors playing at the same stage

During the last decade, a systematic dissection of the research concerning the lysosomal machinery of neuronal tissues (so far solely regarded as serially built degradative compartments) has set in motion better understanding of the endosomal-autophagic pathways. Presently, the general consensus is that broad crosstalk between early endosomes, multivesicular bodies (MVB), autophagosomes, amphisomes and lysosomes (all the effector structures) play a distinct role in the above pathways.

Historically, MVB have been described morphologically by means of ultrastructural analysis. Using electron microscopy (Figure 1), MVB can be visualized as spherical organelles limited by a continuous single layered membrane encompassing several round or ellipsoidal small vesicles within the inner matrix [1]. The outer membrane along with the innermost ones immersed within a clear matrix have a thickness similar to the plasmalemmal structure, possibly due to their endocytic derivation (approximately 5.2 nm) [2,3].

In neurons, MVB are most frequently found in cell bodies and dendrites. Less commonly they may also be found in normal axons or axon terminals in vivo [4-6]. The distribution, biogenesis and multiple functions of MVB in neuronal compartments has been extensively described in a recent review [7]. In this context, MVB maturation into [8,9] or fusion/sorting with lysosomes [10-12] aims to target internalized ligand/receptors, unnecessary proteins and toxins for clearance via degradation or export to the extracellular space or neighbouring cells. The achievement of such a complex goal does not rule out the interaction with other closed compartments called autophagosomes that are essential for the constant turnover of long-lived proteins, cellular macromolecules and whole organelles [13-16]. Autophagic activity, also referred to as type II programmed cell death [17], follows defined steps usually termed as vesicle nucleation and expansion, maturation and finally degradation orchestrated by phagophores, autophagosomes/amphisomes and autolysosomes respectively [18,19]. Therefore, we will concentrate our focus on the experimental data that demonstrates how vesicular trafficking encompassing both autophagosomes and MVB ultimately leads to convergence between the endocytic and autophagic pathways (Figure 2). Regarding this specific matter, this review underscores to what extent developmental neural homeostasis and neurodegenerative phenomena rely on concerted action between MVB, autophagosomes and related organelles.

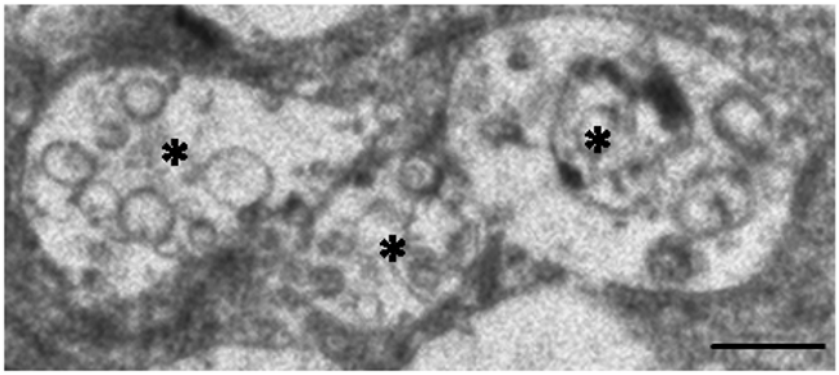

Figure 1. Ultrastructural features of three distinct multivesicular bodies (MVB, asterisks) containing inner vesicles of different size in neuron from a fetal neuronal cell culture. Scale bar = 300 nm.

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Development

The presence of MVB has been ascertained in dendrites and cell bodies of developing (embryonic) neurons [20-23]. However, the involvement of MVB in growth formation and membrane trafficking necessary for neurite outgrowth in developing neurons of the cortex and hippocampus [24-26] remains ambiguous because the identity of the vesicles as MVB, recycling endosomes, or another type of endosome has not yet been elucidated. Despite this, it has been estimated that there is a higher number of MVB per spine (MVB density) in the hippocampal neurons of younger rats compared to older ones [1].

The dramatic impact of autophagic processes during embryonic development has been recently confirmed [27,28]. In addition, mutations of autophagy regulatory genes (Atg) result in severe developmental perturbation. In mammals, Beclin 1 protein (the orthologue of Atg6) when bound to Ambra1 protein has a crucial role in neural tube formation. In particular, mutations of *Ambra1* seem to be responsible for midbrain/hindbrain pathologies [29]. Finally, it should be noted that very few studies refer to the concomitant presence of MVB and autophagosomes, especially concerning their number and distribution. This makes it difficult and speculative to argue about quantitative and/or qualitative differences in CNS neurons at early versus late developmental stages.

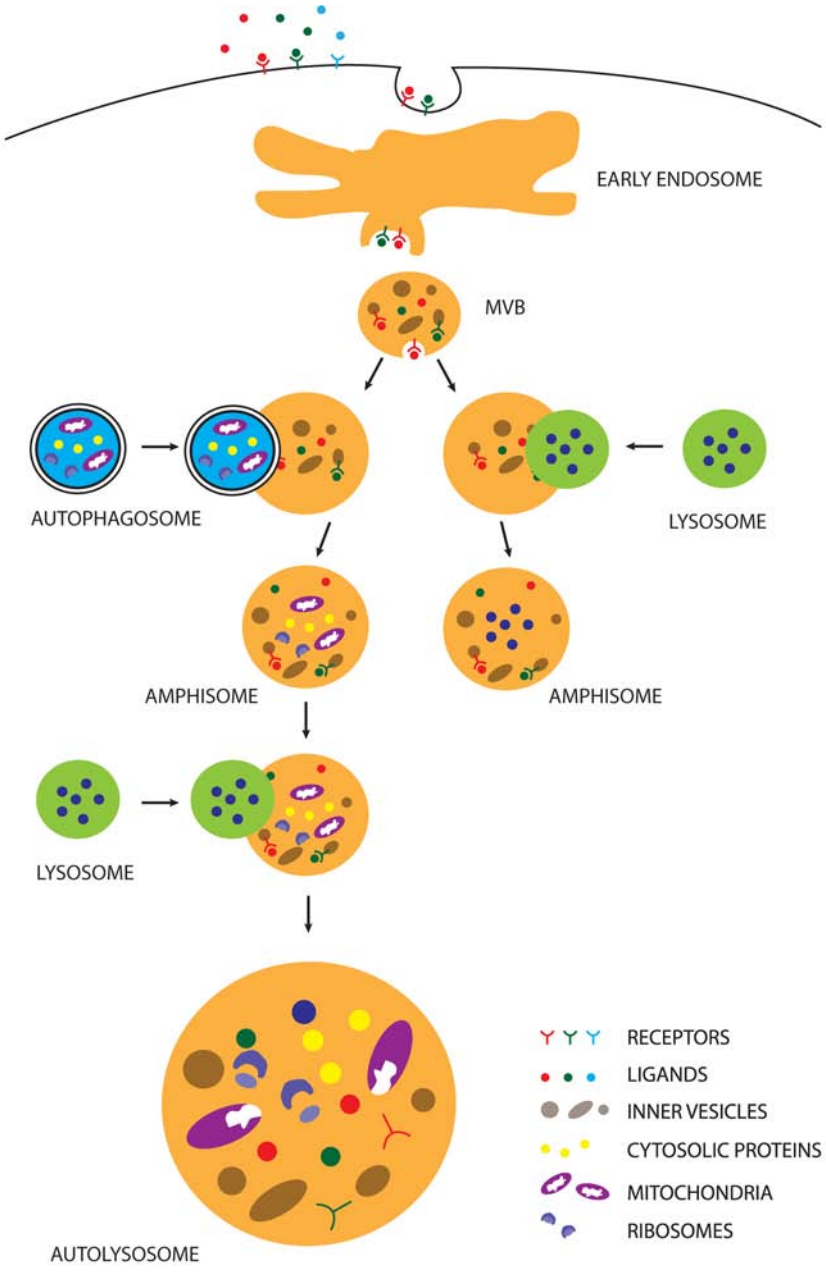

Figure 2. Schematic drawing of known and potential interactions between organelles of the endosomal and autophagic/lysosomal pathways. MVB = multivesicular bodies.

Neurodegeneration

Progressive neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are all characterized by massive neuronal loss that is the final outcome of the aggregation and deposition of abnormal proteins. Some research data indicate that both MVB and autophagic vacuoles (AV) are recruited during the course of these pathologies [30]. It has been ascertained that mutations or any kind of alteration targeting the endosomal-lysosomal machinery, with MVB acting at the intersection point, gives rise to defective endosomal sorting and possible neuronal demise [31-35]. Alternatively, some authors have put forward the hypothesis of a neuroprotective role for MVB/late endosomes and lysosomes by means of taking over the aberrant proteins [36]. The critical role of MVB in AD has already been indicated in an early study [37] on the neurites of senile plaques where "giant" and numerous MVB were found in the cortical neurons of AD patients [38]. More recently, Takahashi et al. [39] have provided direct evidence for the accumulation of amyloid beta 42 (Aβ42) taking place immediately before the appearance of dystrophic neurites and suggesting the MVB as temporary docking stations for the harmful peptide. On the other hand, this phenomenon overlaps

with an increase of immature AV due to the impairment of AV transport to lysosomes and relative failure of lysosome clearance [40].

In PD, the hallmark pathology is represented by the accumulation of the protein alpha-synuclein that is also the major component of Lewy body inclusions. In this case, it has been demonstrated that an overexpression of alpha-synuclein induces autophagy impairment mediated by the inhibition of GTPase Rab-1A, which is required for the autophagosome formation [41]. Therefore, at first glance, the autophagic process could exert beneficial effects because both macroautophagy and chaperone mediated autophagy (CMA) are actively triggered for alpha-synuclein degradation in neurons [42-44]. In contrast with the above findings, it has been reported that massive cell loss can be observed in the dopaminergic neurons in postmortem PD brains as a result of the increase in autophagic activity determined by oxidative stress [45]. Furthermore, it has recently been published that in patients affected by FTD and ALS, functional MVB are required for autophagic clearance of protein aggregates. This was highlighted by the fact that autophagy degradation is arrested in cells depleted of endosomal sorting complex required for transport (ESCRT) subunits or in cells expressing a mutant form of charged multivesicular body protein 2B (CHMP 2B) that has a severe impact on ubiquitin-protein aggregates [31,40]. An additional study [35] has indicated a positive correlation between *CHMP 2B* mutations and FTD manifestation. Therefore, we can draw the conclusion that concerning the different neurodegenerative syndromes, the boundaries between pathogenic autophagy associated with neuronal loss and basal neuronal autophagy remain undefined.

Conclusions

There is an increasing body of data advancing the concept that prolonged perturbance or unbalance in the activity ratio between ESCRT and autophagic machinery with MVB at the intersection point can switch the basal autophagic pathway necessary for the establishment of developmental homeostasis to the irreversible stages of neurodegenerative events.

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