

A New Role for ApoE-Not Only in Alzheimer's Disease?

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Abstract

The by far most relevant genetic risk factor for Alzheimer's disease is possession of the allelic epsilon 4 variant of the cholesterol transporter apolipoprotein E. The apolipoprotein E allelic polymorphism impacts on both histopathological hallmarks of Alzheimer's disease, the intraneuronal tau-aggregates forming neuropil threads and neurofibrillary tangles and the extracellular plaques built-up by beta-amyloid. Autophagy is a crucial process in neurons and plays a central role in cellular degradation of protein aggregates including those composed of tau or beta-amyloid. We discuss available evidence for a role of the apolipoprotein E polymorphism in autophagy.

Keywords: Alzheimer's disease; Autophagy; Apolipoprotein E polymorphism

Introduction

Apolipoprotein E (apoE) [1] shuttles cholesterol between cells on both sides of the blood brain barrier but independently regulated on each side. In brain, apoE attached to a HDL-like particle represents the major cholesterol transporter, moving cholesterol synthesized by astrocytes to neurons. In humans, apoE is encoded by an allelic polymorphism. Each of the three major protein variants (termed apoE2, apoE3 and apoE4) is composed of 299 amino acids and differs only in either of two amino acids at residues 112 and 158, respectively. About 1 of 5 individuals carries at least one copy of the allelic variant epsilon 4 coding for apoE4. Interestingly, possession of this allelic epsilon 4 variant represents by far the commonest genetic risk factor for Alzheimer's Disease (AD) afflicting both familial and sporadic AD [2]. Extracellular deposition of beta-amyloid and formation of insoluble aggregates of protein tau are considered as key features in the development of AD. They may evolve decades before overt clinical symptoms, such as dementia [3,4]. And apoE's polymorphism impacts differentially on these diagnostic neuropathological hallmarks, i.e. apoE4 promotes them whereas apoE2 seems to delay their occurrence when compared to apoE3, respectively [5-7]. Notably, formation of beta-amyloid deposits depends on the presence of apoE as indicated by experiments with apoE-deficient mice [8].

Although publication of epsilon 4 as a risk factor for AD by Alan Roses' group is dated back to 1993, the mechanism by which variants of ApoE impact on the development and course of AD is enigmatic until now. Because neurons are post-mitotic cells they need to effectively handle harmful conditions. Thus, for neuronal survival an appropriate autophagic flux is crucial, i.e. a balanced formation and degradation of autophagosomes [9,10]. These organelles shuttle cellular molecules and even organelles (such as mitochondria) designated to be degraded by lysosomes in a process called (macro) autophagy [11,12]. Notably, removal of protein aggregates require properly working autophagy because the main other cellular institution to degrade proteins, the proteasome, cannot degrade such aggregates by principle. However, about two-thirds of cellular proteins can be handled by either of the two degradation machineries, allowing a given cell to shift the degradation-designated molecules between the machineries when one is challenged by increased degradation demands. In addition, the degree of post-translational modification, such as phosphorylation, determines which route is taken to degrade a molecule. Thus, changes in the degree of phosphorylation, which is considered to be an early event in the development of tau aggregation, may already challenge the

capacity of the autophagic machinery even in the absence of already formed tau-aggregates. Quite interestingly, inhibition of autophagy impair indirectly the proteasomal pathway with subsequently a further accumulation of degradation-designated molecules [13]. However, once aggregates have been formed, they must be routed to the lysosome. And then the actual capacity of the autophagic machinery to degrade such aggregates may determine what will happen. Consequently, if the autophagic flux becomes hampered enough, molecules to be degraded only by lysosomes may accumulate and build-up inside the neuron. Thus, smaller tau aggregates may eventually develop into so-called neuropil threads and neurofibrillary tangles. And here, apoE's new role may come into play.

ApoE and Autophagy

Basically, two principal cellular conditions have to be considered when thinking on mechanistic pathways by which apoE- isoforms may differentially modulate autophagy. One is given if there was an apoE-isoform related difference already in the basal autophagic flux. In this case no further co-factor is required to display differences in genotype-related differences in the autophagic flux. The second condition is related to challenging conditions. In this scenario, ApoE-related differences will only emerge if there are challenging conditions bringing about genotype-related differences in the capacity to adapt the autophagic flux to the challenge. Late endosomal/lysosomal disturbances have been reported in Alzheimer neurons since long and to occur already in early phases of AD [14-17] but no direct role for apolipoprotein on autophagy was addressed.

Quite recently, Simonovitch and colleagues reported genotype-related effects in autophagy for astrocytes [18]. However, the data

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provided are not easy to interpret. For example, using immortalized astrocytic cell lines, under non-challenged conditions ('basal condition') they found a higher LC3-II/LC3-I-ratio in Western blots in apoE3 than in apoE4 cells suggesting for a genotype-related and 'basal' difference. But, in apparent contradiction, there was no genotype-related effect detected when structures considered to be autophagosomes were quantified in histological preparations on both levels, light- and electron microscopically. Further, a widely used protein signal allowing monitoring lysosomal degradation is the level of p62, a molecule specifically degraded by the lysosome *via* autophagy. Remarkably, they do not see a genotype-related difference in the ratio p62/actin. No difference was seen in extracts from cultured primary astrocytes with different genotypes when LC3-II levels were expressed as the LC3-II/actin ratio. LC3-II/LC3-I-ratios for cultured primary astrocytes were not provided. Our own until yet unpublished experimental data from humanized cultured primary astrocytes (derived from targetedly replaced apoE3 and apoE4 mice (Taconics)) do not reflect any genotype-related differences under basal conditions, neither for the LC3-II/LC3-I-ratio, the LC3-II/actin ratio nor the p62/actin ratio. This confirms the data on primary astrocyte cultures of Simonovich et al., but contradicts in part those of their cell line data. Thus, primary cultured astrocyte may not show differences while astrocytic cell lines may (even though the data by Simonovitch on these cells are not unequivocally).

Notably, human apoE can be expressed by neurons. Simonovitch and colleagues did not analyze neurons. Interestingly, our preliminary data on cultured primary neurons (also obtained from the Taconic animals) display differences under basal conditions, with a higher LC3-II/LC3-I ratio and LC3-II/actin ratio in apoE3 compared to apoE4 animals. This difference is in line with the reported difference seen in the astrocytic cell line [18]. And as to be expected, our mixed glial/neuronal cultures show in their protein extracts a measure at level in-between pure astrocytic and pure neuronal cultures. These data suggest a cell-type specific effect. It is tempting to speculate that this cell-type specificity (primary astrocyte versus neuron) is related to a more crucial role of autophagy in post-mitotic cells.

In order to get *in vivo* data, we examined mice brains at postnatal day 7, i.e. at an age comparable to the age of the cultured cells. Not surprisingly regarding the findings from the primary mixed cell cultures, we did not see any genotype related difference neither in LC3-II/LC3-I-ratios nor in p62/actin ratios, which holds true also for brains of 24 months old mice. This is interesting because the relative contribution of the autophagic pathway in proteostasis (i.e. autophagic versus proteasomal degradation) is considered to be higher in aged brain and, therefore, an apoE- genotype-related effect on basal autophagy should be easier to detect in older animals.

What remains, however, is the question how can ApoE-genotype-related differences in basal autophagy at all evolve? Considering that each isoform only differs from one other in just one amino acid exchange out of 299 composing each protein. Parcon et al. [19] suggest a different direct and specific binding of the apoE-isoforms to the CLEAR (coordinated lysosomal expression and regulation) motif, resulting in differences in the levels of autophagy-related gene expression. This point to the possibility that there are cell-type specific differences in the effect of apoE isoforms on basal autophagy due to differences in the protein expression levels of autophagy-related genes.

It is well known that starvation, leading to reduced ATP-levels, induces autophagy. Interestingly, ATP-concentrations seem to differ between apoE3 and apoE4 genotypes when examining dissociated brain cells of targeted-replaced mice, showing significantly lower

ATP levels in ApoE4-carriers [20]. Thus, these animals seem to live in a condition which is in favor of a stimulated autophagy, which in turn may lead to an increased autophagic flux. Unfortunately, the authors did not monitor autophagy in their study. Proteasomal activity, however, was found to be similar in the different apoE-genotypes. This suggests that there is no direct effect of the apoE-genotype on the other major protein degradation system. Interestingly, Simonovitch and colleagues did also experiments in which they have grown their astrocytic cell lines under starving conditions. Signs of a genotype-related response to an induction of autophagy were seen, suggesting differences in the autophagic flux, with apoE3 performing better than apoE4. However, again, the data are not easy to interpret, because of the fact that apoE isoforms may be expressed at different protein levels in cell lines [21]. Unfortunately, Simonovitch and colleagues did not give characteristics of the specific astrocytic cell lines they have used. Their cell lines were reported to secrete different amounts of either apoE3 and apoE4 when expressed as ng apoE per mg protein in cell lysates. On average, apoE4 lines showed lower relative levels than apoE3 lines, with only 63% of the value for example when looking on the highest expressing lines, respectively [21]. Likewise, brain of homozygous E3 individuals contains a higher apoE protein-level, than homozygous E4 individuals [22]. Thus, differences in flux may simply result from differences in apoE-protein levels which may further mediate their effects *via* CLEAR-binding.

A recent paper suggested a role of apoE-isoforms in a tauopathy independent of AD [23]. The authors used a P301S mouse model of frontotemporal dementia with parkinsonism located on chromosome 17 (FTDP17) expressing the human apoE variants. A gene expression difference in some autophagy-related genes was shown when comparing apoE-knock-out, apoE3 and apoE4. Unfortunately, no protein levels of the respective gene-coded proteins were determined-which makes it difficult to interpret the potential functional relevance.

Of course an important information will come from originary human cells. A first hint was given in microarray transcriptome studies of human laser-microdissected astrocytes from Braak-staged autopsy brains of different apoE genotype. In isocortical stages (i.e. V and VI) apoE4 has significantly lower expression of the autophagy related gene ATG7 in comparison to apoE3 but not in earlier stages [24]. However, no other autophagy related genes (ATGs) were found changed, pointing to the possibility that there is no gross shift in astroglial autophagy (as in experimental studies outlined above). In a pilot study, we analyzed post-mortem human brains seeing apoE-genotype-related differences in LC3-II/LC3-I ratio only in brains with a higher stage of Braak's neurofibrillary tangle staging. This seems at first glance to be in line with the outlined animal data: either there is no difference in basal autophagy or non-neuronal cells mask neuron-specific differences (as discussed above) due to a neuron/glia ratio which is about 1:10 in humans. The observed difference in higher Braak-stages may then only be seen in Western blots when there is a challenge of the autophagic flux. It is conceivable that higher Braak stages may reflect such a challenge, because tau aggregates can either use it more than to full capacity or even block the lysosomal degradation machinery with a subsequent increase of LC3-II signal. Likewise, the lysosomal machinery may be at its limits due to beta-amyloid. Beta-amyloid aggregates are reported to be cleared apoE-isoform dependently [25] and are known to impair lysosomal function isoform-differentially [26]. At second glance, however, in human brain tissue, apoE4 bearing individuals of higher Braak-stages for tauopathy show increased contents of LC3-II in brain instead of lower or similar levels as observed in cultured astrocytic cell lines and mouse neurons.

Conclusion

Taken together first indication is given that apoE seems to have a role in autophagy and this role may depend on both its isoform and cell type. Apart from the fact that most of the above reviewed data require confirmation by independent groups and with increased number of experiments, it is unclear what the molecular pathways then will be by which this role is performed. But in case that autophagic flux indeed differs apoE-isoform dependently in various cell types including neurons, this will have impact on the course of diseases being associated with intraneuronal protein aggregates - such as tauopathies. The autophagic flux can be modulated at numerous steps. Identification of the apoE-dependent steps may help to treat Alzheimer's disease and other less common tauopathies.

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