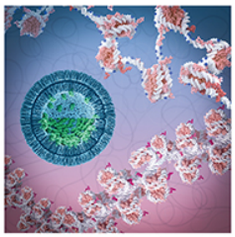
Autophagy



Autophagy

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/kaup20

Hunting out the autophagic problem in Huntington disease

Karolina Pircs, Roger A. Barker & Johan Jakobsson

To cite this article: Karolina Pircs, Roger A. Barker & Johan Jakobsson (2022) Hunting out the autophagic problem in Huntington disease, Autophagy, 18:12, 3031-3032, DOI: 10.1080/15548627.2022.2069438

To link to this article: https://doi.org/10.1080/15548627.2022.2069438

Published online: 03 May 2022.

AUTOPHAGY
2022, VOL. 18, NO. 12, 3031–3032
https://doi.org/10.1080/15548627.2022.2069438

Taylor & Francis
Taylor & Francis Group

AUTOPHAGIC PUNCTUM

Hunting out the autophagic problem in Huntington disease

Karolina Pircs [a,b,c], Roger A. Barker[d], and Johan Jakobsson [a]

[a]Laboratory of Molecular Neurogenetics, Department of Experimental Medical Science, Wallenberg Neuroscience Center and Lund Stem Cell Center, BMC A11, Lund University, Lund, Sweden; [b]HCEMM-SU, Neurobiology and Neurodegenerative Diseases Research Group, Budapest, Hungary; [c]Institute of Translational Medicine, Semmelweis University, Budapest, Hungary; [d]Wellcome-MRC Cambridge Stem Cell Institute & John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, Forvie Site, Cambridge, UK

ABSTRACT

Huntington disease is an inherited, progressive, incurable neurodegenerative disorder that primarily affects cells in the brain. Although the genetic basis for this condition has been known for nearly 30 years, how this causes disease is still unresolved. Of late there has been increasing evidence suggesting that dysfunction in macroautophagic/autophagic pathways may contribute to cellular dysfunction and death. In our recent work we highlight more precisely how and where this problem might arise in this pathway using directly reprogrammed neurons.

ARTICLE HISTORY
Received 22 March 2022
Revised 10 April 2022
Accepted 19 April 2022

KEYWORDS
Autophagy; CRISPR; huntington disease; induced neurons; lentiviral vector; macroautophagy; neurodegenerative diseases; reprogramming

Main

Huntington disease (HD) is an inherited chronic neurodegenerative disorder that typically presents in midlife with a combination of involuntary movements, cognitive deficits, and psychiatric disturbances as well as sleep problems. It is caused by a CAG repeat expansion in the first exon of *HTT* (huntingtin) and although HTT is ubiquitously expressed in the human body, most of the pathology is found in the brain.

Mutant HTT (mHTT) has a propensity to aggregate and form insoluble protein inclusions and with time this causes cellular dysfunction and death. Impairments in autophagy have been linked to HD and pre-clinically it has been shown that boosting autophagy, using genetic or pharmacological means, reduces aggregate burden and provides benefit in various HD models, such as transgenic mice. These promising pre-clinical findings have led to the initiation of clinical trials using a drug repurposing approach to activate autophagy in HD. However, it remains unclear exactly how and why autophagy is impaired in patients with HD. To date, most of the work looking at this has used animal models that poorly replicate the human disorder – in particular they tend to use young animals with very long CAG repeats (often >100 CAG; in the clinic most patients have a repeat length of 39–45). Thus, new experimental approaches that better recapitulate the human disease are needed.

In our recent study [1] we developed a new human model system to study autophagy impairments in HD using "aged" neurons (Figure 1). This model is based on the direct reprogramming of skin fibroblasts to induced neurons (iN) through the overexpression of two transcription factors, POU3F2/BRN2 and ASCL1, in combination with shRNA-knockdown of REST. We found, using this approach, that fibroblasts obtained from HD patients reprogrammed with the same efficiency as the ones obtained from healthy controls and that they retained the age of the donor cells as confirmed using global DNA-methylation patterns in iNs and the Horvath epigenetic clock. Interestingly, we found a significantly increased biological age in the HD-iNs compared to the Ctrl-iNs, similar to what has been found in postmortem HD brains. These data suggest that iNs are suitable for the study of alterations in cellular processes in later onset human neurodegenerative disorders because they are a relevant cell type (neurons), display signs of aging and represent the genetic background of the affected patients.

To identify which cellular pathways are altered in HD, we next performed a global transcriptomics and proteomics analysis of these iNs. The proteomic analysis revealed that HD-iNs display alterations in the AMPK pathway, which is a key energy sensor that promotes autophagy directly by phosphorylating autophagy-related proteins in the, e.g., MTORC1, ULK1, and PIK3C3/VPS34 complexes. We also found a downregulation in the key autophagy regulator BECN1. This suggests that there are alterations in autophagy in HD-iNs which are not seen at the mRNA-level suggesting that these changes are mostly due to post-transcriptional mechanisms. We also found very limited evidence of autophagy alterations in the original fibroblasts, suggesting that neurons are particularly vulnerable to these HD-linked changes.

To complement the proteomics findings, we performed western blot analysis and automated microscopy analysis after immunocytochemistry (ICC) of the HD-iNs, with a focus on autophagy mechanisms. We found clear evidence of impaired autophagy in HD-iNs, including a reduction in LC3B conjugation and an increase in SQSTM1/p62 levels. Notably, the ICC analysis demonstrated that these impairments were largely found in the cell processes, the neurites, where we found an accumulation of LC3B-, SQSTM1- and LAMP1-positive puncta.

CONTACT Johan Jakobsson johan.jakobsson@med.lu.se Laboratory of Molecular Neurogenetics, Department of Experimental Medical Science, Wallenberg Neuroscience Center and Lund Stem Cell Center, BMC A11, Lund University, BMC A11 221 84 Lund, Sweden

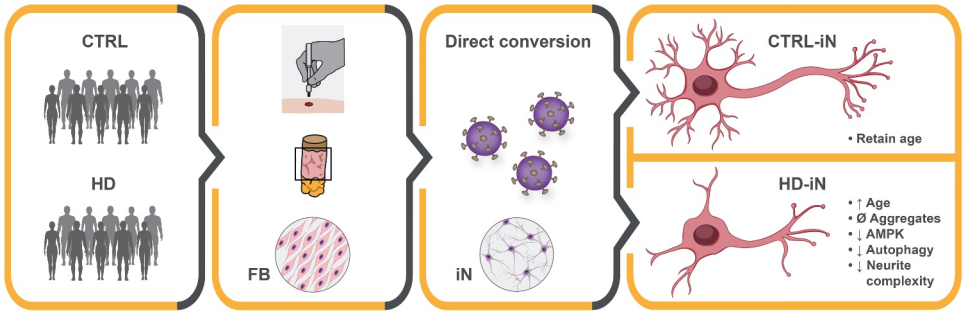


Figure 1. To generate induced neurons (iNs), fibroblasts are harvested from Huntington disease patients and healthy controls. Fibroblast (FB) cultures are established from skin biopsies and then directly reprogrammed to induced neurons using an all-in-one lentiviral reprogramming vector, which overexpresses two transcription factors, POU3F2/BRN2 and ASCL1, in combination with shRNA-knockdown of REST. While both Ctrl and HD-iNs retain the epigenetic aging signatures of the donors, HD-iNs show an accelerated aging compared to the controls. We found a cell-type specific impairment of the AMPK pathway, which is only present in the HD-iNs but not in the parental FB cells. This alteration is not detected at the mRNA level, suggesting that these changes are mostly due to post-transcriptional mechanisms. We find a clear impairment of autophagy mainly detected on a subcellular level in the neurites, which also show a reduced complexity in the HD-iNs compared to the Ctrl-iNs.

All of this suggests that there is a subcellular, neurite-specific impairment of autophagy in HD-iNs, which is characterized by the accumulation of autophagosomes.

Furthermore, when we manipulated autophagic activity in HD-iNs using wortmannin, bafilomycin A_1 or rapamycin we found that autophagic flux is altered. Autophagosomes are formed in HD-iNs at normal rates but fail to be transported to the cell body for degradation. Notably, rapamycin treatment, which provides a benefit in several animal models of HD, fails to resolve the phenotype in HD-iNs. Rather, rapamycin treatment increases the amount of trapped autolysosomes in the neurites. Thus, it appears that the cellular machinery in HD-iNs is working at a reduced rate and indicates that treatment strategies should aim to enhance autolysosme transfer and degradation, rather than autophagy activation per se.

The next question we sought to address was why autophagy is impaired given no protein aggregates could be seen in the HD-iNs. One possibility is that normal *HTT* itself is involved in the regulation of autophagy and to investigate this, we used CRISPRi vectors to silence both alleles of *HTT*. We found that silencing of *HTT* in Ctrl-iNs results in distinct autophagy impairments, confirming the link between HTT and regulation of autophagy. When mHTT/HTT is silenced in HD-iNs, we find evidence for the rescue of some of the autophagy linked phenotypes – but mHTT/HTT silencing also results in additional new autophagy impairments. Thus, the autophagy impairment in HD-iNs appears to be due to a combination of a loss-of-function and gain-of-function mechanisms of mutant HTT.

Finally, we investigated the cellular consequences of impaired autophagy in HD-iNs. Autophagy has in several recent studies been linked to neurite formation and we found that HD-iNs display a significant reduction in neurite complexity. This phenotype is similar to what we found when treating Ctrl-iNs with inhibitors of autophagy. In addition, starvation results in a further reduction of neurite length in HD-iNs, likely due to the failure of autophagy initiation. Thus, these results link the abnormal neurite morphology in HD-iNs to impairments in autophagy. Similar alterations in neurite complexity can also be seen in postmortem tissue from HD patients.

In summary, in our recent study we developed a novel model system that recapitulates many of the key features of HD. Our results demonstrate that autophagy impairments are a key cellular phenotype in aging HD-neurons. This impairment is characterized by a late-stage block of autophagy where autolysosomes are trapped in neurites and fail to be transported to the soma for degradation. This finding should aid in the design of future therapeutic strategies to activate autophagy in HD.

Disclosure statement

J.J. is a co-inventor of the patent application PCT/EP2018/ 062261 owned by New York Stem Cell Foundation.

ORCID

Karolina Pircs http://orcid.org/0000-0001-8281-4785
Johan Jakobsson http://orcid.org/0000-0003-0669-7673

Reference

[1] Pircs K, Drouin-Ouellet J, Horvath V, et al. Distinct subcellular autophagy impairments in induced neurons from Huntington's disease patients. Brain. 2021 Dec 22; awab473. doi:10.1093/brain/awab473.