MALIN KNOCKOUT MICE SUPPORT A PRIMARY ROLE OF AUTOPHAGY IN THE PATHOGENESIS OF LAFORA DISEASE

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Lafora disease (LD), a fatal neurodegenerative disorder characterized by intracellular inclusions called Lafora bodies (LBs), is caused by recessive loss-of-function mutations in the genes encoding either laforin or malin. Previous studies suggested a role of these proteins in regulating glycogen biosynthesis, in glycogen dephosphorylation and in the modulation of intracellular proteolytic systems. However, the contribution of each of these processes to LD pathogenesis is unclear. Here we review our recent finding that dysfunction of autophagy is a common feature of both laforin- and malin-deficient mice, preceding other pathological manifestations. We propose that autophagy plays a primary role in LD pathogenesis and is a potential target for its treatment.
Lafora disease. Lafora progressive myoclonus epilepsy, or LD (OMIM 254780), is an autosomal recessive neurodegenerative disorder resulting in severe epilepsy and death. First described in 1911 by the Spanish neurologist Gonzalo Rodríguez Lafora in patients with “teenage-onset myoclonus epilepsy with dementia,” the presence in the cytoplasm of neurons of PAS-positive intracellular inclusions, nowadays called LBs, is a hallmark of this disorder. LBs are essentially composed of insoluble, starch-like and poorly-branched glycogen molecules, called polyglucans, and their very low protein content (6%) distinguishes LD from other neurodegenerative disorders with inclusion bodies. As LD progresses, patients develop dementia with apraxia, aphasia and visual loss, leading them to a vegetative state and death, usually within the first decade from the onset of the disease. Although nonneurological symptoms are rare in LD, LBs are found in the tissues of most patients, and it is still unclear whether they cause the pathology or are simply a consequence.

To date, LD-causing mutations have been identified in two genes, EPM2A (60%) and EPM2B (40%), encoding respectively laforin, a dual-specificity protein phosphatase with a carbohydrate-binding domain, and malin, an E3 ubiquitin-ligase. Early observations that patients carrying loss-of-function mutations in laforin or malin are indistinguishable, anticipated the subsequent demonstration that both proteins organize into a complex: the critical player in LD pathogenesis. However, it is still unclear how these mutations produce the disease.

Pathogenic mechanisms in Lafora disease. Since laforin or malin mutations, both in humans and in animal models, correlate with accumulation of LBs in many tissues, it was proposed that glycogen metabolism is disturbed in
LD. This is supported by evidence that the laforin-malin complex downregulates glycogen synthase (GS) and the protein targeting subunit to glycogen (R5/PTG) of protein phosphatase 1 through malin-dependent ubiquitination and proteasome degradation. However, different studies were unable to identify alterations in the levels or activities of proteins implicated in glycogen metabolism, perhaps because its modulation by the laforin-malin complex is a subtle process. In this regard, it was recently shown that lowering glycogen synthesis by R5/PTG depletion reduces LBs, and that decreased laforin levels correlate with elevated glucose uptake, both observations supporting an impact of glycogen synthesis in LD pathogenesis.

A remarkable observation was the recognition that laforin is a glycogen phosphatase. Based on these and other data it was proposed that, by suppressing excessive glycogen phosphorylation, laforin prevents the formation of LBs. Importantly, this activity does not require malin, which makes unclear the role of the laforin-malin complex in the process. Also, the inhibition of the activity of laforin by glycogen binding and the ubiquitination of laforin by malin for degradation are difficult to accommodate in this hypothesis.

Finally, it has been demonstrated that, in the absence of the laforin-malin complex, unfolded proteins and other proteolytic substrates accumulate. Laforin may be acting here as an ancillary protein bringing proteasome or autophagy substrates to malin for ubiquitination or, upon ubiquitination, directing them to degradation. In addition, the laforin-malin complex may directly regulate the activity of the proteolytic systems (see below).

**Analysis of autophagy in laforin- and malin-deficient mice.** We
previously observed compromised autophagosome formation in laforin-deficient human fibroblasts and mice (MEFs and liver) and that overexpression of laforin increases LC3-II levels. Therefore, we proposed that laforin activates autophagy. Our studies also suggested that the protein(s) responsible for this modulation were in the MTOR pathway, upstream of TSC1/TSC2.

We have now extended these observations to other tissues, particularly brain, and to malin-deficient mice, demonstrating similar defects in autophagy. This is consistent with the identical phenotypes of LD patients with laforin or malin mutations, and illustrates that it is a lack of the laforin-malin complex, due to either laforin or malin loss, that impairs autophagy.

Autophagy dysfunction precedes other pathological manifestations, suggesting that this event is critical in the early stages of LB formation. We, therefore, propose (Fig. 1) that a combination of both impaired autophagy and glycogen synthesis dysregulation results in the formation of LBs and in the neurological and behavioral abnormalities of LD. This is likely exacerbated, when laforin is lacking, by uncontrolled glycogen hyperphosphorylation.

**Future research.** If our interpretations are correct, impaired autophagy would be one of the primary causes of accumulation of polyglucans in LBs. Therefore, we will analyze in our LD mice models the therapeutic benefits of enhancing autophagy.

A clear conclusion from our work is that impairment of autophagy in both LD mice models is a consequence of the lack of laforin-malin complexes. In this respect, our earlier report using laforin-deficient mice indicating that autophagy dysfunction could be in part explained by activation of MTOR is in apparent
conflict with our failure to demonstrate a similar result in brains and in malin-deficient mice. Additional experiments will determine the precise molecular mechanisms underlying autophagy impairment in LD. At present, we can only state that autophagy dysfunction is likely due to an altered MTOR-independent mechanism and that the observed MTOR activation occurs only in certain tissues and is exclusively related to laforin-deficiency.

While autophagy probably regulates LB formation, it is possible that other consequences of defective autophagy, beyond glycogen metabolism, are relevant in LD pathogenesis. In fact, not all neurons undergoing degeneration in LD have visible LBs, suggesting that they are not the cause of LD, but one of their many pathological manifestations. Probably, other autophagy substrates, including proteins, lipids and damaged mitochondria, accumulate in LD. The contribution to LD pathogenesis of these and other processes, like endocytosis, synaptogenesis and neural plasticity, potentially altered by a compromised autophagy, will be considered in future investigations.
Acknowledgements

Work in the author’s labs was supported by the Spanish Ministry of Science and Innovation (SAF2008-00226 to SRdeC; BFU2010-16031 to PB; BFU2008-00186 to EK; SAF2008-01907 to PS; and SAF2010-18586 to JMS), the Red de Biobancos del FIS (RD09/0076/00011 to CN), the Fondo de Investigación Sanitaria (PI10/02628 to CN; PI070023 to MPS), the Fundación Marato TV3 (Ref. 100130 to PS, EK and SRdeC) and the Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER). AMGC holds a fellowship from the Fundación Conchita Rábago.
Figure 1. Pathogenic mechanisms in Lafora disease

Functions of the laforin-malin complex would be to avoid the generation of polyglucans or, if already formed, to prevent their accumulation, degrading them before they become pathogenic. Glycogen hyperphosphorylation and dysregulation of glycogen synthesis, produced by loss-of-function mutations in either laforin or malin, are two non-mutually exclusive possibilities that may concur together to accumulate LBs. Also, the laforin-malin complex could be involved in the clearance of nascent LBs and other substrates, targeting them to autophagy or to the ubiquitin-proteasome system (UPS). Alternatively, it could enhance the activities of both proteolytic systems. Concerning this, only autophagy is impaired in both LD mice models (laforin- and malin-deficient), and this alteration precedes the formation of LBs. Therefore, autophagy is a potential target for the pharmacological treatment of the disease, for example with drugs that increase an autophagic response. The figure also depicts that neurodegeneration can occur without detectable formation of LBs, which can simply be one of the manifestations of the disease and not the cause.
Glycogen hyperphosphorylation
Dysregulation of glycogen synthesis
Impairment of intracellular proteolysis (Autophagy, UPS)

LB formation
Neurodegeneration

LAFORA DISEASE