Interrupting synoviolin play at the ER: a plausible action to elevate mitochondrial energetics and silence obesity

Meghan S Soustek & Pere Puigserver

Obesity is a global concern, which has been linked to increased risk for cardiovascular disease, type 2 diabetes, atherosclerosis, non-alcoholic fatty liver, and cancer. In this issue of *The EMBO Journal*, Fujita *et al* (2015) describe the role of an endoplasmic reticulum (ER)-resident E3 ubiquitin ligase, synoviolin, and its ability to control body weight and energy expenditure by targeting PGC-1 β , a transcriptional modulator of mitochondrial oxidative metabolism.

See also: H Fujita *et al* (April 2015)

The ER ensures correct protein sorting and folding in the secretory pathway and carries out several metabolic processes. Accumulation of misfolded proteins or lipid overload results in ER stress that alters protein sorting and secretion and triggers an adaptive response to correct protein misfolding (Ron & Walter, 2007). A molecular pathway that may be involved in ER stress signaling and obesity is the ER association degradation (ERAD) pathway, which consists of E3 ubiquitin ligases that degrade misfolded proteins. Chronic lipid overload and ER protein misfolding in pathological conditions such as obesity lead to ER stress, although the underlying mechanisms are not fully understood. Some studies have proposed that cellular increases in saturated free fatty acids produce ER stress and inflammatory processes in adipocytes (Hotamisligil, 2010). Other studies showed that ERAD proteins are increased in the subcutaneous fat of obese individuals (Sharma *et al*, 2008). Previously, the E3

ubiquitin ligase synoviolin, SYVN1 (or HRD1, DER3), was shown to play a role in the ERAD pathway (Bays *et al*, 2001; Yagishita *et al*, 2008). SYVN1 is upregulated in rheumatoid synovial cells, and mice deficient in SYVN1 are resistant to inflammation and developing collagen-induced arthritis (Gao *et al*, 2006). Fujita *et al* (2015) now provide novel insights into SYVN1, showing that it controls body weight and mitochondrial biogenesis through degradation of PGC-1 β , a member of the PGC-1s transcriptional coactivator family that promotes oxidative metabolism by increasing mitochondrial mass (Puigserver & Spiegelman, 2003).

With limited options available for the prevention or treatment of obesity, there has been increasing interest in studying energy metabolism in adipocytes to obtain further insight into underlying mechanisms that regulate energy balance. Here, the authors generated adipose-specific SYVN1 KO mice and used several models of genetic and diet-induced obesity. The mice displayed a marked reduction in body weight with a similar food intake, indicating that SYVN1 regulates whole body energy balance in adipocytes. Mitochondria maintain energy metabolism, and although these organelles are not overly abundant in white adipose tissue (WAT), accumulating evidence suggests that accelerated respiratory and thermogenic rates in white adipocytes are sufficient to increase energy expenditure and protect against obesity (Tseng *et al*, 2010). To investigate the possible molecular mechanisms whereby SYVN1 controls body weight and energy balance, Fujita *et al* performed a microarray gene expression

analysis and identified overproduction of several transcripts linked to fatty acid β -oxidation and mitochondrial biogenesis. Moreover, as the PGC-1 coactivator family is known to control this global gene profile (Puigserver & Spiegelman, 2003), the authors investigated whether SYVN1 impinged on the PGC-1 coactivators (PGC-1 α and PGC-1 β). PGC-1 coactivators act as a docking site for other proteins, favoring the assembly of the basal transcription machinery to initiate gene expression. PGC-1 α has been shown to act as a mediator of mitochondrial biogenesis during times of high-energy demands and responds to environmental/physiological stimuli (i.e., cold induction, exercise, caloric restriction). PGC-1 β can also increase mitochondrial biogenesis and can partially compensate for the loss of PGC-1 α in brown fat cells (Uldry *et al*, 2006). The authors show through protein-protein interaction assays that SYVN1 interacts with and ubiquitinates PGC-1 β , causing re-localization to the perinuclear region and ultimately resulting in the degradation of PGC-1 β protein. Furthermore, they show that PGC-1 α does not interact with SYVN1, demonstrating specificity between the PGC-1 family members. These experiments elegantly demonstrate that PGC-1 β is negatively regulated by SYVN1, providing a plausible mechanism by which this E3 ubiquitin ligase controls mitochondrial biogenesis and energetics.

Previously, the same group has also shown that the chemical compound LS-102 inhibits the ubiquitination activity of SYVN1 in a dose-dependent manner with an IC₅₀ of 35 μ M *in vitro* and acts as a selective

inhibitor that can also suppress rheumatoid arthritis in a mouse model (Yagishita *et al.*, 2012). Here, they examined the effect of LS-102 in mouse embryonic fibroblasts and in wild-type and *db/db* mice. LS-102 treatment correlated well with adipose-specific SYVN1 KO data. Thus, in treated mice, PGC-1 β ubiquitination levels were reduced, mitochondrial numbers were increased, and the mice exhibited decreased body weight and adiposity, and diabetic symptoms were improved. These experiments provide initial pharmacological evidence that SYVN1 E3 ligase activity might be a potential therapeutic target to treat obesity and other associated diseases.

This study highlights the functional cross talk between the ER and mitochondria, suggesting that mitochondrial biogenesis and respiratory function might be intercepted as a result of ER stress (Fig 1). The ER is physically linked to mitochondria via membrane contact sites and can stimulate mitochondrial respiration (Hotamisligil, 2010). Therefore, it is conceivable to speculate that during ER stress, ubiquitination of PGC-1 β might serve as an initial regulatory

point to decrease mitochondrial biogenesis in an attempt to facilitate correct protein folding through the ERAD pathway. However, persistent and chronic ER stress, as observed during obesity, will dampen mitochondrial respiratory rates, facilitating lipid overload and thereby maintenance and progression of obesity.

Finally, this work gives rise to a number of questions. It is still unclear whether and how SYVN1 is regulated during ER stress *in vivo*, particularly within the context of obesity and adipocyte lipid overload. In addition, it will be necessary to assess whether SYVN1 inhibitors interfere with ER protein folding during ER stress, compromising protein sorting and distribution. Along these lines, whether the small molecule LS-102 might disrupt ER homeostasis in different cell types and at what specific concentration should be explored. *In vivo* data from previous work dosed mice with LS-102 at a concentration of 4 mg/kg; however, here, the authors increased the dosage to 50 mg/kg (Yagishita *et al.*, 2012). Future work should focus on assessing potential off target effects at different doses

of LS-102 using SYVN1 KO mice to show the specificity of the chemical compound. Lastly, the precise thermogenic mechanism by which energy expenditure is increased in SYVN1 KO mice is not well defined. The identification of the thermogenic mediators (e.g., uncoupled respiration, ATP hydrolysis, futile cycles), which act in concert with increased mitochondrial biogenesis and fatty acid oxidation, will be critical to understand the cellular energetic mechanisms involved in SYVN1's regulation in obesity. Altogether, these experiments should help in the design of putative new effective and safe approaches in treating obesity and other metabolic or inflammatory disorders.

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Figure 1. ER cross talk to the mitochondria.

Under normal cellular lipid loads and correct ER protein folding (left), ER stress is low and E3 ubiquitin ligases in the ERAD pathway are downregulated or inactive. Based on Fujita *et al.*, inactive synoviolin (SYVN1), an ERAD E3 ubiquitin ligase, leads to increases of the transcriptional coactivator PGC-1 β protein that stimulates mitochondrial biogenesis and oxidative metabolism. This, in turn, causes an increase in energy expenditure and protection against obesity. Cellular lipid overload or ER protein misfolding causes high levels of ER stress and activation of the ERAD pathway (right), and SYVN1, also involved in inflammatory processes, is augmented. Active SYVN1 ubiquitinates and degrades PGC-1 β , dampening mitochondrial biogenesis and energy expenditure that leads to progression of obesity.

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