Hepatic ERAD takes control of the organism

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Upregulation of Endoplasmic Reticulum-Associated Degradation (ERAD) in the liver upon feeding and organismal growth aggravates proteasomal turnover of the transcription factor CREBH and decreases the expression of its target gene, the hepatokine FGF21. Wei *et al* and Bhattacharya *et al* describe the systemic coordination of energy metabolism and organismal physiology mediated by hepatic-specific ERAD function.

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See also: J Wei *et al* (November 2018) and A Bhattacharya *et al* (November 2018)

ERAD is a key quality control mechanism in the cell that recognizes misfolded proteins in the endoplasmic reticulum (ER). These proteins are retrotranslocated for ubiquitylation and proteasomal degradation into the cytosol. The best characterized E3 ligase responsible for the ubiquitylation of luminal and membrane-bound proteins is Hrd-1 coupled to its regulatory adaptor Sel-1L (Wu & Rapoport, 2018). While the molecular basis of ERAD function has been extensively studied in *Saccharomyces cerevisiae* and mammalian cells, its physiological relevance in multicellular organisms is currently poorly explored. Upon ER stress, the ERAD machinery is transcriptionally upregulated by the corresponding unfolded protein response pathway (UPR^{ER}). Conversely, recent findings have described a role of ERAD in feedback regulation of the UPR^{ER}. For example, Ire-1 α , one of the three ER-associated stress sensors, is degraded via ERAD to downregulate stress response. Since the whole-body knockout of Hrd-1 is lethal, little information has been

gathered on its substrates. Therefore, targeted depletion of ERAD opened up a new route to study Hrd-1-dependent degradation pathways. Recent work on tissue-specific regulation mechanisms has already identified additional ERAD substrates important for UPR^{ER}, such as the antioxidant transcription factor Nrf-2 in hepatocytes and the ER luminal lectin Os-9 and the lipoprotein lipase Lpl in adipocytes (Qi *et al*, 2017).

Wei *et al* and Bhattacharya *et al* characterized liver-specific Hrd-1 and Sel-1L knockout mice and identified a key role for ERAD in energy metabolism. The knockout mice displayed growth retardation, female infertility, improved insulin sensitivity, and reduced body weight upon feeding with high-fat diet. These phenotypes resembled those induced by overexpression of fibroblast growth factor 21 (FGF21). Strikingly, serum and hepatic levels of FGF21 were upregulated in Hrd-1 and Sel-1L knockout mice suggesting a direct regulation mechanism (Bookout *et al*, 2013; Bhattacharya *et al*, 2018; Wei *et al*, 2018). FGF21 is a hepatokine, which is involved in the regulation of carbohydrate and fatty acid metabolism in the liver (Fon Tacer *et al*, 2010). Prolonged fasting strongly induces hepatic expression of endocrine FGF21 in mice, which can induce browning of white adipose tissue (Shan *et al*, 2018). Interestingly, animal models of type 2 diabetes and obesity treated with FGF21 displayed reduced body weight, fat mass, and hepatic triglyceride content, and displayed improved glucose tolerance and insulin sensitivity (Nygaard *et al*, 2018; Shan *et al*, 2018). According to its hepatokine function, FGF21 expression is tightly regulated by various transcription factors. Upon ER stress, FGF21 levels are

increased through ER-resident transcription factors Atf-4, Chop, and Xbp-1s, demonstrating a close interplay between ER homeostasis and FGF21 regulation. Atf-4 further activates FGF21 expression upon autophagy deficiency in muscle cells or food deprivation. Fasting-induced FGF21 expression is regulated by transcription factor cyclic adenosine monophosphate-responsive element-binding protein H (CREBH) and peroxisome proliferator-activated receptor alpha (Ppar α) (Nakagawa & Shimano, 2018).

In agreement with the phenotypic correlation, the metabolic phenotypes in both liver-specific Hrd-1 and Sel-1L knockout mice were in part related to FGF21 levels. In fact, proteomics and RNA-sequencing analyses of liver extracts from these knockout mice identified the transcription factor CREBH as a potential target of ERAD in the liver. CREBH is anchored to the ER membrane and transported along the secretory pathway to the Golgi apparatus where it is cleaved off the membrane by site-1- and site-2-specific proteases, which are also involved in cholesterol metabolism. The soluble, active form of CREBH (CREBH-N) subsequently translocates to the nucleus where it is thought to form a complex with Ppar α to induce FGF21 expression (Brown & Goldstein, 1999; Wang *et al*, 2016). Biochemical experiments showed that CREBH directly interacts with the ERAD machinery for ubiquitin-dependent degradation initiated by the E3 ligase Hrd-1 (Bhattacharya *et al*, 2018; Wei *et al*, 2018). While ERAD substrates are usually polyubiquitylated via lysine residues K11 and/or K48 (Locke *et al*, 2014), Wei *et al* suggested that CREBH is modified with K27-linked ubiquitin chains (Wei *et al*, 2018). This

Figure 1. Degradation of CREBH through ERAD regulates hepatic FGF21 level.

During fasting, CREBH is transported to the Golgi where it is subsequently cleaved by the site-specific proteases, site-1 protease (S1P) and site-2 protease (S2P). CREBH-N, the active form of CREBH, translocates to the nucleus where it forms a complex with Ppar α to activate FGF21 expression and further regulate metabolism. During feeding, the hepatic ERAD machinery is upregulated and degrades the transcription factor CREBH resulting in downregulation of FGF21.

finding raises the question why CREBH ubiquitylation is mediated via a non-canonical linkage-type: Is K27-linked ubiquitin chain topology regulated by fasting signals?

The degradation of CREBH plays a crucial role in the feeding and fasting cycle as well as during growth (Fig 1). As a function of organismal energy metabolism, the Hrd-1-Sel-1L complex is upregulated in the liver, which leads to the degradation of CREBH and reduction of FGF21 level. Otherwise, defects in ERAD result in increased CREBH level and FGF21 overexpression. Considering the liver-specific role of ERAD, it will be interesting to further address how Hrd-1 and Sel-1L are upregulated upon metabolic changes and which factors are involved in the feeding

response. A rather intriguing possibility is that ERAD might be directly regulated by changes in food composition. Taken together, Wei *et al* and Bhattacharya *et al* identified a systemic role of ERAD by regulating the abundance of the hepatokine FGF21, which nicely demonstrates the tremendous impact of tissue-specific regulation mechanisms for organismal energy metabolism and physiology. Future studies on similar tissue-specific ERAD pathways will shed light on its physiological relevance related to metabolic and aging-associated diseases.

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