Impact of ER Protein Homeostasis on Metabolism

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Proper regulation of protein homeostasis in a cell is critical for the health of the organism. Proteins, intracellular or secreted, need to be produced and maintained at the right quantity, folded into their three-dimensional conformation with necessary posttranslational modifications, targeted to their correct destinations to insure their optimal function, and degraded efficiently and disposed when needed. The overall process is tightly regulated at many levels to increase or decrease the rate of the synthesis and breakdown of polypeptides. In most cases, failure to properly regulate any one of these control points leads to a dysfunction at the cellular and organismic level resulting in disease.

The endoplasmic reticulum (ER) is a cellular organelle consisting of a vast tubular network, where about one-third of all proteins are synthesized. Proteins secreted to extracellular environment, membrane proteins, and proteins that reside inside the ER lumen are all synthesized and folded into their native conformation in this organelle. Later, most of the posttranslational modifications are completed in the Golgi compartment. Similar to cytoplasmic protein synthesis machinery, ER-mediated protein synthesis involves a complex molecular apparatus to insure the quantity and quality of synthesized proteins (1). ER-mediated protein synthesis and folding represent unique challenges compared with its cytoplasmic counterpart due to the high oxidative environment and high calcium concentrations of the ER lumen (2). Problems arising at the synthesis and folding stages lead to the appearance and accumulation of unfolded proteins in the ER lumen that have to be efficiently cleared using the primary protein degradation machine, the 26S proteasome, which mediates degradation of most of the ER resident as well as cytoplasmic proteins (3). Misfolded proteins, as well as the organelle itself, can also be removed through autophagy (4). Synthesis and degradation machinery interact with each other closely, and it is widely accepted that dysfunction of either of these processes compromises the efficiency of the other. If unfolded proteins appear in the lumen, a cellular adaptation program named the unfolded protein response (UPR) is triggered to increase the folding and degradation capacity of the ER. Once the burden of unfolded proteins is removed, UPR response is silenced to its initial basal state. Prolonged or inappropriate UPR responses are causally linked to various pathologies including neurodegenerative diseases, obesity, and diabetes (5,6).

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Degradation and removal of unfolded proteins from the ER system are specifically named as ER-associated degradation (ERAD). Defects in ERAD are associated with an increasing number of human diseases, including metabolic diseases (7). One plausible mechanism linking ERAD dysfunction to pathological states involves chronic UPR responses resulting from the negative impact of a defective ERAD on the protein synthesis and folding machinery. Consistent with this hypothesis, a large number of ER chaperones, which normally assist protein folding, were shown to be involved in targeting unfolded proteins to ERAD pathway, providing further support that the two processes are simultaneously controlled (8,9).

New evidence of ERAD dysfunction leading to diabetes emerged from a study by Otoda et al. (10), which is reported in this issue of *Diabetes*. The initial leads that prompted Otoda et al. to investigate the involvement of protein degradation dysfunction in obesity and related metabolic diseases came from expression profiling of obese and diabetic human patients. As previously reported in obese liver tissue in mouse models (11), the expression of several members of proteasome-mediated degradation machinery was increased. Interestingly, however, the authors detected a significant decrease in the activity of the proteasome accompanied by the accumulation of ubiquinated proteins in liver tissue in various mouse models of diabetes. The authors suggest that increased expression of components of the proteasome system is a result of feedback cellular adaptation to the decreased proteasome activity.

Otoda et al. next generated a genetic mouse model lacking all three isoforms of the *PA28A* family of proteasome activator genes to test whether compromised protein degradation is causal to metabolic disease. Deletion of *PA28A* genes did, in fact, resulted in significantly decreased proteasome activity and accumulation of ubiquinated proteins in liver tissue lysates. Importantly, this state was accompanied by increased hepatic glucose production even in the presence of high levels of insulin. Experiments, in vivo, also provided support to the presence of hepatic insulin resistance, despite the fact that liver tissue analysis appeared normal except for a rather mild increase in lipid accumulation. However, a more detailed examination of hepatocytes by electronmicroscopy revealed unusual expansion and disorganization of ER membranes in cells lacking PA28A subunits α, β, and γ. This observation prompted the investigators to hypothesize that ER stress maybe the mechanistic link between proteasome dysfunction, insulin resistance, and abnormal glucose metabolism. This is a sound postulate as many independent studies have firmly established that there is chronic ER stress in liver tissue both in experimental models and in humans with obesity and type 2 diabetes (12–14). Furthermore, proteasome dysfunction induced by specific inhibition of hepatic CYP3A has been shown to trigger UPR response in cultured rat hepatocytes (15). Consistently, PA28A-deficient animals displayed increased UPR response, evident by elevated expression of several ER stress indicators such as PERK,

IRE1α, and eIF2α phosphorylation, GRP78 and CHOP accumulation, JNK activation, and the consequential decrease in insulin-induced Akt phosphorylation. These results demonstrate that experimentally induced defects in proteasome activity can be sufficient to cause ER stress and lead to insulin resistance.

Several important questions are raised from these observations to explore and address in the future. It is interesting that Otoda et al. observed further increase in the ubiquinated proteins in PA28A-deficient animals on a high-fat diet. Since there are other proteasome complexes in the cell such as the PA700-containing complexes (3), it is likely that the remaining proteasome activity may also be susceptible to dietary challenges and cues. Moreover, earlier studies have shown defective autophagic responses in the liver of obese mice (16). Autophagy is an important cellular process by which proteins and cellular organelles are degraded in lysosomes and contribute to protein and organelle turnover in the cell. Besides, degradation obviously is not the only control apparatus for protein homeostasis, therefore synthetic pathways might also play a role in this condition. Increased lipid accumulation in hepatocytes might be a consequence of hepatic insulin resistance and ER stress activation. Interestingly, lipoprotein synthesis is regulated by ERAD via degradation of ER resident lipogenic enzyme HMG-CoA (17). It is possible that other ER enzymes of de novo lipogenesis pathway are also ERAD substrates.

Otoda et al. postulate that increased hepatic glucose production may be linked to increased nuclear Foxo1 activity due to inefficient degradation of phosphorylated Foxo1 protein by the proteasome. In this scenario, one would expect accumulation of the phosphorylated form of Foxo1 in the cytoplasmic compartment. Surprisingly, phosphorylated Foxo1 is lower in PA28A-deficient animals compared with wild-type controls suggesting that proteasomal dysfunction might not be the main reason for decreased Foxo1 degradation. Akt phosphorylates Foxo1 upon insulin stimulation, preventing its nuclear transport (18); therefore decreased Akt activity might contribute to inefficient exclusion of Foxo1 from the nucleus and targeting to proteasomal degradation.

And finally, it is not clear if the accumulation of a substrate of UPS in ER lumen, cytoplasm, or even in the nucleus, triggers downstream signaling events. For instance, ubiquinated Huntington protein aggregates and forms cytoplasmic and intranuclear inclusion bodies, which correlate with the progression of Huntington disease. While the contribution of these inclusion bodies to neuronal cell death and disease pathogenesis remains a subject of debate (19), it is quite possible that intermediary misfolded products and their accumulation at levels insufficient to form aggregates, can directly engage ER stress responses. At the moment, there is no clear evidence to support this possibility.

In summary, the results of this study illustrate that obesity correlates with impaired UPS and reduced proteasomal degradation in hepatocytes, thus leading to activation of chronic UPR responses, hepatic insulin resistance, and increased hepatic glucose production. This model is also supported by the findings that treating PA28A-deficient animals with a chemical chaperone phenylbutyric acid (PBA) ameliorated ER stress and increased hepatic insulin response. It is yet unclear whether the finding reported by Otoda et al. indicate defects in overall protein turnover rates or those of specific proteins. This question is of interest since systems approaches have demonstrated diminished protein synthesis in obesity in the obese liver tissue (11).

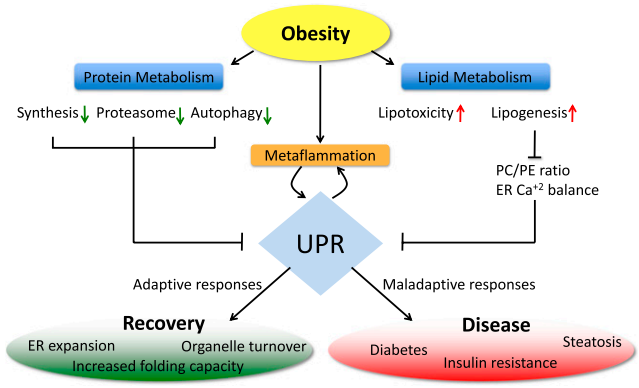

FIG. 1. Disruption of ER protein homeostasis in obesity. Through its impact on protein synthesis, folding, and degradation checkpoints, obesity negatively impacts protein homeostasis of the hepatocyte and liver tissue. ER-mediated protein synthesis is closely linked to degradation pathways (UPS and autophagy) through ERAD and organelle turnover. Disturbances in these critical regulatory nodes disrupt ER homeostasis, lead to chronic UPR response, which in turn initiate metabolic inflammation, disrupt insulin action, glucose metabolism, and hepatosteatosis. These pathology events further compromise ER function and protein homeostasis, thus feeding a vicious cycle. PC, phosphatidylcholine; PE, phosphatidylethanolamine.

Hence the net result of these changes on protein homeostasis is not known at this point and would be interesting to explore.

Most striking finding of Otoda et al. is decreased proteasomal function by metabolic interventions such as increased caloric intake and increased fat content in the diet. What initiates these disturbances in the ER protein synthesis and degradation systems remains a critical future area of research. Increased hepatic lipogenesis (11), circulating fatty acids and other nutrients (20), and exposure to oxidative stress arising from increased oxidative phosphorylation (21) and insulin (22) are some potential candidates that may tip the balance of protein synthesis and degradation systems of the ER.

It is likely that there are close communication systems linking ER protein homeostasis with UPS and autophagy machinery through ERAD and ER turnover processes, respectively. Any disturbance to this delicate balance results in the induction of ER stress, metaflammation, insulin resistance, and steatosis in liver tissue (Fig. 1). Which one of these biological processes receives the first signal from the increased nutrient surplus during obesity remains to be identified. The study by Otoda et al. uncovers an important piece of the puzzle, adds to the model that obesity is accompanied by inherent abnormalities in homeostatic mechanisms that regulate protein turnover, and raises important open questions for future studies.

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REFERENCES

1. Bukau B, Weissman J, Horwich A. Molecular chaperones and protein quality control. Cell 2006;125:443–451
2. Dill KA, Ozkan SB, Shell MS, Weikl TR. The protein folding problem. Ann Rev Biophys 2008;37:289–316
3. Finley D. Recognition and processing of ubiquitin-protein conjugates by the proteasome. Annu Rev Biochem 2009;78:477–513

4. Kruse KB, Brodsky JL, McCracken AA. Autophagy: an ER protein quality control process. Autophagy 2006;2:135–137
5. Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell 2010;140:900–917
6. Marciniak SJ, Ron D. Endoplasmic reticulum stress signaling in disease. Physiol Rev 2006;86:1133–1149
7. Guerriero CJ, Brodsky JL. The delicate balance between secreted protein folding and endoplasmic reticulum-associated degradation in human physiology. Physiol Rev 2012;92:537–576
8. Brodsky JL. The protective and destructive roles played by molecular chaperones during ERAD (endoplasmic-reticulum-associated degradation). Biochem J 2007;404:353–363
9. Nishikawa SI, Fewell SW, Kato Y, Brodsky JL, Endo T. Molecular chaperones in the yeast endoplasmic reticulum maintain the solubility of proteins for retrotranslocation and degradation. J Cell Biol 2001;153:1061–1070
10. Otoda T, Takamura T, Misu H, et al. Proteasome dysfunction mediates obesity-induced endoplasmic reticulum stress and insulin resistance in the liver. Diabetes 2013;62:811–824
11. Fu S, Yang L, Li P, et al. Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. Nature 2011;473:528–531
12. Boden G, Duan X, Homko C, et al. Increase in endoplasmic reticulum stress-related proteins and genes in adipose tissue of obese, insulin-resistant individuals. Diabetes 2008;57:2438–2444
13. Ozcan U, Cao Q, Yilmaz E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 2004;306:457–461
14. Wang Y, Vera L, Fischer WH, Montminy M. The CREB coactivator CRTC2 links hepatic ER stress and fasting gluconeogenesis. Nature 2009;460:534–537
15. Acharya P, Engel JC, Correia MA. Hepatic CYP3A suppression by high concentrations of proteasomal inhibitors: a consequence of endoplasmic reticulum (ER) stress induction, activation of RNA-dependent protein kinase-like ER-bound eukaryotic initiation factor 2alpha (eIF2alpha)-kinase (PERK) and general control nonderepressible-2 eIF2alpha kinase (GCN2), and global translational shutoff. Mol Pharmacol 2009;76:503–515
16. Yang L, Li P, Fu S, Calay ES, Hotamisligil GS. Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. Cell Metab 2010;11:467–478
17. Fisher EA, Ginsberg HN. Complexity in the secretory pathway: the assembly and secretion of apolipoprotein B-containing lipoproteins. J Biol Chem 2002;277:17377–17380
18. Rena G, Guo S, Cichy SC, Unterman TG, Cohen P. Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B. J Biol Chem 1999;274:17179–17183
19. Saudou F, Finkbeiner S, Devys D, Greenberg ME. Huntington acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 1998;95:55–66
20. Hamel FG. Preliminary report: inhibition of cellular proteasome activity by free fatty acids. Metabolism 2009;58:1047–1049
21. Osna NA, Haorah J, Krutik VM, Donohue TM Jr. Peroxynitrite alters the catalytic activity of rodent liver proteasome in vitro and in vivo. Hepatology 2004;40:574–582
22. Sadiq F, Hazlerigg DG, Lomax MA. Amino acids and insulin act additively to regulate components of the ubiquitin-proteasome pathway in C2C12 myotubes. BMC Mol Biol 2007;8:23