Viewpoint

Complexity in Hepatic Insulin Resistance – Unraveling the Role of Ubiquitin-Specific Protease 14 in Protein Homeostasis of Metabolic Transcription Factors

Hepatic glucagon and insulin action are central to the systemic regulation of glucose and lipid metabolism. Fasting hepatocytes undergo glycolysis and gluconeogenesis in response to a high glucagon-to-insulin ratio. Postprandially, hepatocytes halt glucose production and store excess nutrients as glycogen and lipids in response to a low glucagon-to-insulin ratio (Santoleri and Titchenell, 2019). During metabolic disease progression, pancreatic beta cells enhance insulin secretion, leading to hyperinsulinemia (Hudish et al., 2019). This chronic insulin stimulus reduces expression of the insulin receptor at the hepatocyte cell surface (Liu et al., 2022), where blunted phosphorylation of transcription factor forhead box protein O1 (FOXO1) leads to continued nuclear access, thereby enabling glucose production in the postprandial state (Brown and Goldstein, 2008). Still, hepatocytes respond to insulin signaling in this state; they continue to accelerate lipid synthesis rates via the continued phosphorylation and nuclear localization of lipogenic transcription factor sterol regulatory element-binding protein 1C (SREBP-1C), rendering selective insulin resistance (Brown and Goldstein, 2008). As such, hepatic lipid accumulation often accompanies poor glucose regulation in metabolic disease.

In addition to promoting the nuclear translocation of SREBP-1C, insulin signaling prevents proteasomal degradation of this lipogenic transcription factor (Botolin et al., 2006). Ubiquitin-specific protease 14 (USP14) is key to several canonical signaling pathways, including insulin signaling (Wang et al., 2022). Upon insulin stimulation, protein kinase B (Akt) phosphorylates USP14 at Ser432, which activates its C-terminal deubiquitinating activity, preventing proteasomal degradation of protein substrates (Xu et al., 2015). The N-terminal ubiquitin-like domain of USP14, however, activates proteasomal activity when bound to ubiquitinated substrates (Wang et al., 2022). This dual functionality of USP14 both protects protein substrates from degradation and promotes protein degradation (Wang et al., 2022). Dysregulation of USP14 activation and expression is linked to cancer, neurodegenerative diseases, immune responses, and viral infection (Wang et al., 2022). Growing evidence for the role of USP14 in metabolic disease includes the identification of fatty acid synthase (FASN) as a bona fide substrate of USP14, where hepatic overexpression and short hairpin RNA (shRNA) silencing of USP14 accelerates hepatic triglyceride accumulation and improves hepatic steatosis, respectively (Liu et al., 2018). Therefore, studies unraveling USP14’s crystal structure and the pursuit of catalytic inhibitors are of therapeutic interest, including 1-[1-(4-fluorophenyl)-2,5-dimethyl-1H-pyrrol-3-yl]-2-(pyrrolidin-1-yl)ethan-1-one (IU1), which allosterically inhibits USP14 from binding to its ubiquitin substrate (Wang et al., 2018). Interestingly, in mouse models of obesity and insulin resistance (ob/ob and db/db), hepatic proteasome activity is reduced by nearly 50%, leading to an accumulation of ubiquitinated proteins (Otoda et al., 2013); its role in hepatic glucose and lipid metabolism is not well understood. Additionally, experiments in rat H4IIEC3 hepatocytes demonstrate that inhibition of the 26S proteasome with bortezomib concentration dependently increases endoplasmic reticulum (ER) stress markers and reduces insulin-stimulated phosphorylation of Akt. Cotreatment of bortezomib and chemical chaperone phenylbutyric acid (PBA), however, reduces the levels of ER stress molecular markers and im-

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Akt, protein kinase B; ER, endoplasmic reticulum; FOXO1, forkhead box protein O1; IU1, 1-[1-(4-fluorophenyl)-2,5-dimethylpyrrol-3-yl]-2-pyrrolidin-1-ylethanone; siRNA, small interfering RNA; SREBP-1, sterol regulatory element-binding protein 1; USP14, ubiquitin-specific protease 14.
proves the levels of insulin-induced phosphorylation of Akt (Otoda et al., 2013), demonstrating that reducing ER stress that is caused by inhibition of the 26S proteasome can improve hepatic insulin signaling in vitro.

Kamoshita et al. (2022) reveal a significant negative correlation of hepatic USP14 mRNA expression in 21 individuals with and 11 without type 2 diabetes with glucose clearance rate under hyperglycemic clamp conditions (Fig. 1). As such, the authors sought to identify the mechanisms regulating the balance of proteasome activity and the number of ubiquitinated proteins under the control of insulin signaling and USP14. Using native PAGE assays, the authors demonstrate that 2 hours of insulin treatment reduces the accumulation of ubiquitinated proteins in rat H4IIEC3 hepatocytes and in human HepG2 hepatoma cells, which is restored upon inactivation of USP14 with IU1 (Kamoshita et al., 2022) (Fig. 1). Moreover, small interfering RNA (siRNA)-mediated knockdown of Usp14 significantly increases the levels of ubiquitinated proteins compared with insulin treatment alone (Fig. 1). Interestingly, the response to insulin and IU1 cotreatment is not maintained in murine Hepa 1-6 cells, where ubiquitinated protein levels are unchanged by insulin treatment (Kamoshita et al., 2022). Next, the authors sought to determine if insulin-activated USP14 also reduces ubiquitinated proteins by enhancing proteasome activity or by modulating ER stress. In-gel assays reveal that inhibition of USP14 activity with IU1 does not prevent insulin-mediated increases in 20S proteasome activity in both rat H4IIEC3 hepatocytes and human HepG2 cells (Kamoshita et al., 2022) (Fig. 1). The 26S proteasome, however, is not significantly impacted by insulin treatment or cotreatment with IU1 in any of the three cell lines tested (Kamoshita et al., 2022) (Fig. 1). When assessing markers of ER stress, immunoblot analyses in H4IIEC3 hepatocytes reveal that IU1 partially attenuates insulin-induced increases in CCAAT/enhancer-binding protein homologous protein (CHOP) protein expression levels (Fig. 1); however, this effect was not replicated with Usp14 siRNA treatment (Kamoshita et al., 2022). Taken together, these experiments demonstrate that insulin reduces the accumulation of ubiquitinated proteins by activating the deubiquitinating activity of USP14 and by increasing the 20S proteasome activity independent of USP14 (Fig. 1).
Next, the authors demonstrate that inhibiting USP14 with IU1 significantly increases the ratio of insulin-stimulated nuclear SREBP-1 to cytoplasmic SREBP-1 protein levels in H4IIEC3 hepatocytes to a greater extent than insulin treatment alone (Kamoshita et al., 2022). This response, however, was not replicated with Usp14 siRNA treatment. Despite this increased nuclear SREBP-1 abundance, IU1 treatment does not change the mRNA abundance of SREBP-1 target proteins including Fasn, Acaca, and Scd1 compared with insulin treatment alone in H4IIEC3 hepatocytes (Kamoshita et al., 2022). By contrast, IU1 cotreatment significantly downregulates the mRNA expression of rate-limiting gluconeogenic enzymes Pck1 and G6pc in H4IIEC3 hepatocytes to a greater extent than insulin alone (Kamoshita et al., 2022). This downregulation is not replicated by Usp14 siRNA treatment. Unlike in H4IIEC3 hepatocytes, IU1 treatment in HepG2 cells does not lower the expression of PCK1 mRNA; it also increases G6PC mRNA expression (Kamoshita et al., 2022).

In this study, Kamoshita et al. (2022) perform careful experiments, including the evaluation of IU1 treatment alone, with insulin, and in the presence of proteasome inhibitor bortezomib and liver X receptor agonist T0901317 as controls. Although the results obtained from cotreatment with IU1 are not always replicated by siRNA-mediated knockdown of USP14, the inclusion of the latter is critical for interpreting the deubiquitinating role of USP14 under the control of insulin signaling. Similarly, the inclusion of rat, human, and murine hepatocyte cell lines (although not always showing consistent results) provides a strong guide for species-dependent differences in future preclinical experiments. Additionally, future experiments probing changes in mRNA expression of all SREBP-1C and FOXO1 targets are of interest and should also include unbiased approaches. Since USP14 confers opposing actions on proteasomal protein degradation (Zhang et al., 2022), experiments evaluating proteasomal activity concomitantly with ubiquitination levels as well as the ubiquitination state of proteins of interest are required for understanding the impact of this tug-of-war–like mechanism.

This study reveals an intriguing complexity to the control of hepatic glucose and lipid metabolism. Transcription factor phosphorylation triggers either nuclear access or exclusion, yet the ubiquitination status, the activity of both the proteasome and the deubiquitinating enzyme USP14 also dictate its stability within the nucleus. The mechanism underlying the increased nuclear abundance of SREBP-1C upon insulin and IU1 cotreatment, however, remains unclear. Future experiments involving catalytic inhibition, mutagenesis of the Ser432 phosphorylation site, and genetic manipulation of USP14 could examine the nuclear stability of SREBP-1C and FOXO1 in the context of insulin resistance, as well as their targets, including regulators of hepatic lipoprotein secretion apolipoprotein C-III and microsomal triglyceride transfer protein. Additionally, evaluating the phosphorylation of USP14 and thus activation in response to insulin treatment, perhaps over a time course since prolonged insulin treatment induces degradation of the insulin receptor and cellular insulin resistance (Liu et al., 2022) would facilitate determining the contribution of USP14 activation to metabolic disease. Moreover, the relation of USP14 and its protein substrates to ER stress during obesity, where markers are upregulated in murine adipose and liver but not muscle tissue (Ozcan et al., 2004), and identifying the targets of the corresponding reduced capacity for transcription and translation merit further investigation.

Overall, Kamoshita et al. (2022) provide important correlations between USP14 mRNA expression and metabolic parameters in individuals with type 2 diabetes. The molecular work provides clues to understanding the regulation of USP14 in hepatocytes in the context of insulin sensitivity, insulin resistance, and timing of drug administration concerning fasting and postprandial periods, which will be critical for the development and careful selection of candidate small molecule inhibitors. Given the involvement of USP14 in neurodegeneration and immune responses as well as autophagy (Wang et al., 2022), targeting the hepatocyte specifically and assessing cellular homeostasis will be essential to limit off-target effects.

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References


