Glypican-4 is a new comer of adipokines working as insulin sensitizer

Obesity is associated with major adverse health outcomes, such as type 2 diabetes, dyslipidemia, hypertension, cancers and so on. These pathologies originate from the common basis of insulin resistance. In the past 20 years, adipocyte biology has unveiled the mechanism linking between obesity and insulin resistance. In this process, the most important factors are adipokine secretion and chronic inflammation in adipose tissues. Adipokine hypothesis elegantly elucidated obesity-induced insulin resistance in which secretory bioactive molecules from adipocytes, referred to as adipokines, could directly regulate the insulin sensitivity of the remote insulin sensitive organs including the liver and skeletal muscle. Deregulated adipokine secretion from the expanded adipose tissue of obese individuals contributes to the development of systemic insulin resistance and metabolic diseases. In 1993, leptin was identified as the first adipokine, which was secreted from adipocytes, and regulated food intake and energy expenditure in the hypothalamus. After this discovery, a series of molecules were identified as adipokines. For example, pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-6 were found to be insulin resistance-inducing adipokines. In contrast, adiponectin turned out to be an adipokine that ameliorated insulin resistance.

Recently, macrophage infiltration to adipose tissue was recognized in obesity and type 2 diabetes. Such a low-grade inflammation in adipose tissue was found to be tightly associated with obesity-related metabolic diseases. Adipocytes are thought to secrete chemokines that attract monocytes when they increase triacylglycerol storage. Recruited monocytes then become activated pro-inflammatory adipose tissue macrophages, which robustly secrete inflammatory cytokines and chemokines (e.g., TNF-α, interleukin-6, monocyte chemoattractant protein-1)[1]. They further promote adipose tissue inflammation, and are released to the circulation and induce insulin resistance as adipokines in the liver and skeletal muscle. Thus, adipokine secretion and adipose tissue inflammation are the two major mechanisms that are tightly associated with each other, and coordinately contribute to insulin resistance in obesity.

Glypicans are a family of heparan sulfate proteoglycans that are linked to the external surface of the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor. They have an N-terminal cysteine-rich domain, and heparan sulfate glycosaminoglycan chains are attached to a C-terminal domain near the anchor at the plasma membrane. Glypicans can bind a variety of soluble and insoluble ligands. In addition, they are shed from the cell surface into extracellular space by the lipase-mediated cleavage of a GPI anchor. These suggest that glypicans can play a role not only in the cell membrane, but also in the extracellular environment including the circulation and remote tissues. In general, glypicans are thought to contribute to cellular proliferation and tissue growth by modifying cell signaling pathways of wingless-type MMTV integration site family members (Wnts), Hedgehogs, fibroblast growth factors and bone morphogenetic proteins. In mammals, the glypican family has six members (glypican-1 to glypican-6). In 2006, glypican-4 was reported to be expressed at the higher level in intra-abdominal epididymal adipose tissues compared with in subcutaneous fat tissues in mice using microarray analysis[2]. However, in humans, this gene was more highly expressed in subcutaneous adipose tissue than in visceral adipose tissue[2]. In addition, there was a very strong correlation of glypican-4 expression with body mass index (BMI) and waist-to-hip ratio (WHR). In this case, the correlation in the two depots was in opposite directions, with decreasing glypican-4 expression in subcutaneous adipose tissue with increasing BMI and WHR, and increasing glypican-4 expression in visceral adipose tissue with increasing BMI and WHR[2]. In the following study in 2012, Ussar *et al.*[3] in the same research group found that glypican-4 was a new adipokine that enhanced insulin signaling by direct interaction with the insulin receptor independent of the GPI anchorage (Figure 1). This interaction occurs with the unoccupied insulin receptor, and stimulation by insulin disrupts the interaction between glypican-4 and the insulin receptor. Such an insulin sensitizer working through direct interaction with insulin receptor is novel. Overexpression of glypican-4 or the addition of recombinant ectodomain of glypican-4 enhanced insulin signaling in cultured adipocytes, whereas depletion of glypican-4 reduced insulin receptor phosphorylation and the following downstream signaling. In adipocytes, glypican-4 plays an important role for adipocyte differentiation through insulin-mediated CCAAT/enhancer binding protein-β (CEBPβ) phosphorylation, which is essential for transactivation of CEBPα and peroxisome proliferator-activated receptor-γ (PPARγ), the key transcriptional factors required for adipocyte differentiation. In addition, the released ectodomain of glypican-4 is also suggested to enhance insulin signaling as an adipokine in the liver and skeletal muscle (Figure 1), although such definite data in the whole body were not yet presented in that study. Taken together, glypican-4 is the first-identified adipokine that affects insulin sensitivity in proteoglycans.

Ussar *et al.*[3] showed that circulating glypican-4 levels positively correlated with

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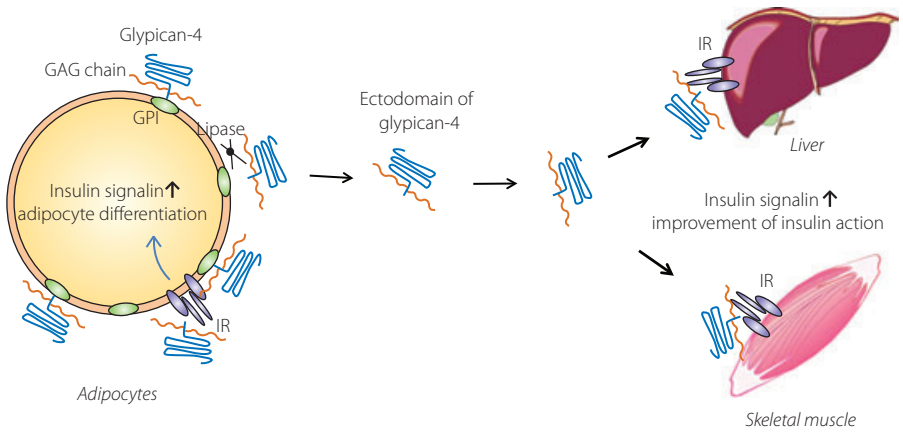

Figure 1 | Glypican-4 is a new type of adipokine that promotes adipocyte differentiation and enhances insulin receptor signaling in the liver and skeletal muscle. Glypican-4 is linked to the cell surface of adipocytes by a glycosylphosphatidylinositol (GPI) anchor, and is released from the cell surface to the extracellular space by an enzymatically-regulated process by GPI-lipase. The released ectodomain of glypican-4 from the adipocytes to the circulation is supposed to enhance insulin receptor signaling through direct interaction with the insulin receptor in adipocytes, the liver and skeletal muscle as an adipokine. GAG, glycosaminoglycan; IR, insulin receptor.

BMI and insulin resistance in humans. These results at a glance prompt us to suppose that glypican-4 might cause insulin resistance in obesity. However, they speculated that increased circulating glypican-4 observed in obesity could be a novel regulatory mechanism by which fat acts to counteract insulin resistance and decrease insulin demand. Thus, decreased circulating glypican-4 level observed in severe obesity in males (BMI > 30) could be failure of compensation, and cause further insulin resistance and accelerate diabetes progression. If so, glypican-4 can be a unique compensator of insulin resistance in obesity. In addition, glypican-4 also has the possibility of being a new type of drug in the treatment of type 2 diabetes. However, several questions remain to be elusive in this study. Why are the expression patterns of glypican-4 in visceral adipose tissues and subcutaneous adipose tissues during the progression of obesity different? Why are serum levels of glypican-4 different between males and females? At the same time, why doesn't the serum level of glypican-4 reflect the expression in visceral fat in females, although it does reflect it in males? This might be responsible for the wide and high expressions of glypican-4 in other tissues besides adipose tissue. In addition, what is the signal that increases glypican-4 expression in adipose tissue? Does the insulin sensitizer, thiazolidinedione, increase glypican-4 expression? Does a systemic administration of glypican-4 enhance insulin signaling and ameliorate insulin resistance? Detailed investigations of glypican-4-deficient mice or adipocyte-specific glypican transgenic mice seem to be very useful and interesting for further understanding the physiological role of glypican-4 in whole-body glucose and energy metabolism.

Recently, glypican-4 was shown to bind Wnt3a and Wnt5a, and enhance both pathways[4]. Given that both Wnt signaling pathways are relevant to adipogenesis, glypican-4 might also affect adipocyte differentiation through Wnt signaling, as well as enhanced insulin receptor signaling[3]. Furthermore, adipose tissue macrophages of obese and type 2 diabetic patients express Wnt5a, which has pro-inflammatory characters[5]. In fact, Wnt5a activated c-Jun-N-terminal kinase 1, which triggered a pro-inflammatory response in adipocyte and macrophages[6]. Serum levels of Wnt5a are also elevated in obese subjects[7]. Considering that the glypican-4 ectodomain released from the cell surface inhibits Wnt5a signaling[4], glypican-4 might also regulate insulin sensitivity by decreasing a pro-inflammatory state through Wnt signaling in adipose tissue. In anyway, the knowledge of glypican-4 function in glucose metabolism is still very limited. Further studies are indispensable for unveiling the whole mechanism of glypican-4 in obesity and diabetes.

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