Identification of hepatokines involved in pathology of type 2 diabetes and obesity

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Abstract. Many researchers pay attention to novel secretory factors, such as adipokines or osteokines, secreted by the tissues that were not formerly recognized as classical endocrine organs. The liver also contributes to the onset of various kinds of pathologies of type 2 diabetes and obesity by producing and releasing secretory proteins “hepatokines.” By using the information of gene expression in human livers, we rediscovered selenoprotein P (SeP) and leukocyte cell-derived chemotaxin 2 (LECT2) as hepatokines involved in the onset of glucose intolerance. SeP was previously recognized as a selenium transport protein, but we revealed that SeP causes insulin resistance in the muscle and liver. SeP also reduces VEGF signal transduction in vascular endothelial cells, contributing the impaired angiogenesis in diabetes. Importantly, SeP impairs health-promoting effects of exercise training by suppressing reactive oxygen species (ROS)/adenosine monophosphate-dependent protein kinase (AMPK) pathway in the skeletal muscle through its receptor low-density lipoprotein receptor-related protein 1 (LRP1). LECT2, previously-reported as a neutrophil chemotactic protein, promotes skeletal muscle insulin resistance in obesity. Further studies are necessary to develop new diagnostic or therapeutic procedures targeting hepatokines to combat type 2 diabetes or obesity.

Key words: Hepatokines, Selenoprotein P, Leukocyte cell-derived chemotaxin 2, Insulin resistance

Introduction

Currently, many studies pay attention to novel secretory factors derived from tissues that were not previously recognized as classical endocrine organs. The liver maintains glucose homeostasis by glucose release and glycogen storage. Notably, the liver is also the site for the production of various secretory proteins. In fact, patients with severe liver cirrhosis usually receive replacement therapy with liver-derived secretory proteins such as albumin. Additionally, most of clinicians diagnose various diseases by measuring blood levels of liver-derived secretory factors such as albumin or coagulation factors. In fact, we have previously reported that genes encoding secretory proteins are abundantly expressed in the livers of humans by using comprehensive gene expression analyses [1]. Because the human liver releases numerous kinds of secretory factors, we thought that the identification of liver-secreted factors might not be enough.

Based on these findings, we hypothesized that the liver may contribute to the development of various kinds of pathologies observed in type 2 diabetes and obesity, through the production of secretory proteins, termed “hepatokines”. Since about fifteen years before, we have identified hepatokines involved in the pathologies of type 2 diabetes and obesity and have shed light on the previously-unknown pathophysiological significance of hepatokines overproduction (Fig. 1).

Identification of Selenoprotein P as a Hepatokine That Induces Insulin Resistance in Type 2 Diabetes

To screen for new hepatic secretory proteins involved in insulin resistance, we used the data of comprehensive analyses of gene expression profiles in human livers. We found out a negative correlation between hepatic selenoprotein P mRNA levels and the metabolic clearance rate of glucose, indicating that the elevation of hepatic selenoprotein P mRNA levels were positively connected with the severity of insulin resistance in humans [2].

Selenoprotein P (SeP encoded by the SEPP1 or
SELENOP gene) is a secretory protein abundantly expressed in human blood. SeP is primarily produced by the liver [3]. SeP contains 10 selenocysteine residues, and functions as a selenium supply protein [4]. SeP attenuates oxidative stress status by acting as an antioxidative enzyme and by supplying selenium to target tissues. However, the role of SeP in the regulation of glucose metabolism or insulin sensitivity was formerly unknown.

To examine the physiological roles of SeP in glucose metabolism in vivo, we treated C57BL/6J mice with purified human SeP. Glucose and insulin tolerance tests revealed that injection with physiological doses of SeP induced glucose intolerance and insulin resistance in normal mice. In contrast, SeP knockout mice fed high fat and high sucrose diet showed improvement of glucose intolerance and insulin resistance in glucose and insulin loading tests. Our study reveals that elevation of circulating SeP contributes to the development of hyperglycemia in type 2 diabetes by inducing insulin resistance [2].

**Hepatokine Selenoprotein P Disturbs Effects of Exercise Training through LRP1**

Physical exercise has various beneficial effects in people. However, not all people derive equal amount of improvement of metabolic health from exercise. An early clinical trial reported that about 20% of patients with diabetes mellitus received poor hypoglycemic effects to regular exercise therapy [5]. A lot of early reports suggest that some people are suffering from “exercise resistance” and they derive limited benefit from the physical exercise. To date, however, secretory factors that cause exercise resistance are not fully understood.

Exercise-induced acute generation of reactive oxygen species (ROS) not only causes oxidative damage, but ROS also function as signaling molecules to induce beneficial molecular adaptations by exercise training [6]. For example, adenosine monophosphate-dependent protein kinase (AMPK) and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α, encoded by Ppargc1a), both of which play a major and central role in exercise-mediated skeletal muscle adaptations, are activated by acute generation of ROS. Notably, growing clinical evidence suggest that supplementation with antioxidants, such as vitamin C, rather abolishes exercise-induced molecular alterations and limits the positive or beneficial effects of exercise training in humans.

Based on previous reports suggesting the involvement of acute generation of ROS in the effects of exercise, we hypothesized that excessive amounts of the hepatokine SeP with anti-oxidative capacity causes “exercise resistance” by suppressing exercise-induced ROS acute generation in the skeletal muscle.

First, we subjected SeP-deficient mice to a high-fat diet (HFD) and regular exercise training. After exercise training for 1 month, SeP-deficient mice showed higher aerobic exercise capacity in a running endurance test. The glucose-lowering effect of insulin was higher in trained Sepp1-deficient mice, indicating that deficiency of SeP enhances the responsiveness to regular exercise training in mice. In the experiments to mimic exercise training in cultured myotubes, treatment with purified SeP suppressed H$_2$O$_2$-stimulated AMPK phosphorylation.
and mitochondrial DNA content. SeP reduced mRNA levels of genes expressed downstream of AMPK, such as Ppargc1a, in the presence of H$_2$O$_2$. Knockdown for AMPKα subunits by small interfering RNA completely abolished the inhibitory actions of SeP on Ppargc1a expression in C2C12 myotubes. Taken together, these results strongly suggest that the hepatokine SeP impairs the effects of exercise training by suppressing ROS/AMPK/PGC-1 pathway in the skeletal muscle.

To identify the uptake receptor of SeP in the skeletal muscle, we transfected C2C12 mouse myotubes, a representative cellular model of the skeletal muscle, with siRNA that were specific to the genes encoding two abundant LDLR family members. This was because previous reports showed that LDLR family proteins, such as megalin, function as SeP receptors in the testis or kidney [7, 8]. Knockdown of Lrp1 (low-density lipoprotein receptor-related protein 1), but not of Ldlr (low-density lipoprotein receptor), reduced SeP-induced Gpx1 mRNA induction in C2C12 cells. Also, the inhibitory effects of SeP on the expression of Ppargc1a were completely abolished by knockdown of Lrp1. Furthermore, knockdown of Lrp1 canceled the inhibitory effect of SeP on H$_2$O$_2$-induced AMPK phosphorylation in C2C12 myotubes. These results indicate that LR1P functions as the functional and uptake receptor of SeP, at least, in cultured mouse myotubes.

Next, we generated muscle-specific knockout mice for LR1P by using muscle creatine kinase Cre expressing mice. Muscle-specific knockout mice for LR1P showed decreased uptake of injected SeP in the skeletal muscle. The suppressive action of SeP on exercise-induced AMPK phosphorylation was also decreased in the muscle of muscle-specific knockout mice for LR1P. After HFD feeding for 1 month, muscle-specific knockout mice for LR1P also exhibited a higher aerobic exercise capacity in exercise endurance test and improved insulin sensitivity in insulin loading test. Taken together, these results strongly suggest that LR1P functions as an uptake receptor of SeP in the skeletal muscle from mice.

Our study concluded that overproduction of hepatokine SeP causes exercise resistance by impairing exercise-induced molecular adaptations in the skeletal muscle through its receptor LR1P [9]. The current findings suggest that screening for inhibitors of the SeP-LR1P axis may identify exercise-enhancing drugs to treat physical inactivity-associated diseases such as type 2 diabetes.

**Identification of LECT2 as a Hepatokine That Is Over-Produced in Obese Condition and Induces Skeletal Muscle Insulin Resistance**

Non-alcoholic fatty liver disease may play a central role in the development of insulin resistance in obesity. Hence, to identify hepatokines involved in obesity-induced insulin resistance, we performed liver biopsies in humans with obesity and type 2 diabetes and performed a comprehensive analysis of gene expression profile. As a result, we found out a positive correlation between hepatic LECT2 mRNA levels and body mass index (BMI), indicating that elevated hepatic LECT2 mRNA levels were associated with severity of obesity in humans [10].

Leukocyte cell-derived chemokatin 2 (LECT2) is a secretory protein identified as a novel neutrophil chemotactic protein [11]. LECT2 (in humans encoded by the LECT2 gene) is expressed preferentially in human adult and fetal liver cells. To date, however, the role of LECT2 in the development of obesity and insulin resistance induced by over-nutrition was not formerly established.

We examined the role of LECT2 in the development of insulin resistance in mice. A glucose or insulin loading test revealed that Lect2$^{-/-}$ mice showed lower blood glucose levels after glucose or insulin injection. Lect2$^{-/-}$ mice exhibited an increase in insulin-stimulated Akt phosphorylation in skeletal muscle, but not in the liver or adipose tissue. These results indicate that deficiency of hepatokine LECT2 increases insulin sensitivity in the skeletal muscle in rodents.

To assess the effect of the hepatokine LECT2 on insulin signaling in vitro, we transfected C2C12 myotubes with a plasmid expression vector encoding mouse LECT2. LECT2 protein was secreted from C2C12 cells transfected with LECT2 expression plasmid into culture medium. The cells transfected with the LECT2 vector showed a decrease in insulin-stimulated signal transduction and an increase in basal c-Jun-N-terminal kinase (JNK) phosphorylation, a negative regulator of insulin signaling. Our research is the first to show that the over-production of hepatokine LECT2 contributes to the development of muscle insulin resistance in obesity [10].

**Conclusions and Future Perspectives**

We have discovered SeP and LECT2 as hepatokines that cause insulin resistance and hyperglycemia. After the publication of our reports on SeP and LECT2, many researchers have paid a lot of attention to hepatokines involved in the regulation of glucose metabolism. Many secretory factors, including adropin, fetuin A/B, fibro-
blast growth factor 21 (FGF21), and sex hormone-binding globulin (SHBG), were identified as hepatokines linked to the induction of metabolic dysfunction [12]. Importantly, our research elucidates that abnormal production of hepatokines participates in the development of various pathologies observed in type 2 diabetes, and suggest that hepatokines may be targets to treat or diagnose the pathologies in over-nutrition-associated diseases. In fact, high-throughput screening for low molecular inhibitors of hepatokines production is now ongoing. Additionally, treatment with neutralizing antibodies against human SeP was reported to improve insulin resistance in animal models with obesity and diabetes [13]. For the diagnosis of hepatokines-related pathologies, we have already established the rapid measurement system of blood concentrations of human SeP. We strongly anticipate that novel diagnostic or therapeutic procedures targeting hepatokines to combat over-nutrition-related diseases such as type 2 diabetes and obesity would be developed.

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Conflict of Interest

The author has no competing interests to declare.

References