

## New Aspects in Obesity and Diabetes: Involvement in Regucalcin, a Suppressor Protein of Cell Signaling

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Obesity and diabetes (type 1 and 2 diabetes) are currently a major health problem worldwide with growing in prevalence. The incidence of metabolic disease, including type 2 diabetes with obesity, has increased to epidemic levels. Obesity and diabetes induce secondary diseases with various pathophysiologic states, which are important in clinical aspects including cardiovascular disease, neural disturbance, kidney disease, osteoporosis and cancer [1-6]. Obesity is based on stimulation of adipogenesis. Bone marrow mesenchymal stem cells are multipotent cells, which among other cell lineages, and give to differentiate into adipocytes, osteoblasts, chondrocytes and myoblasts [1]. This occurs through cross talk between complex signaling pathways including those derived from bone morphogenic proteins, wingless-type MMTV integration site (Wnt) proteins, hedgehogs, delta/jagged proteins, fibroblastic growth factors, insulin, insulin-like growth factors, and transcriptional regulators of adipocyte and osteoblast differentiation including peroxisome proliferators-activated receptor-gamma (PPAR $\gamma$ ) and runt-related transcription factor 2 (Runx2) [1-3]. Insulin, which is secreted by feeding, stimulates adipogenesis from bone marrow mesenchymal stem cells. In addition, bone marrow adiposity and mature adipocytes with obesity greatly produces tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), an inflammatory cytokine [4]. This TNF- $\alpha$  may cause insulin resistance that leads to type 2 diabetes.

Various hormones and cytokines, which include leptin, adiponectin, insulin, epinephrine, cortisol, glucagon, TNF- $\alpha$  and other factors, are well known as key molecules that relate to obesity and diabetes. Disturbance of these factors may play an important role in pathophysiologic conditions of obesity and diabetes. In addition, it has been proposed that regucalcin, a suppressor protein of intracellular signaling systems [7], may be a key molecule in obesity, diabetes and osteoporosis. Regucalcin has been demonstrated to stimulate adipogenesis in mouse bone marrow cell culture *in vitro* [8], suggesting an involvement as a stimulatory factor in adipogenesis.

Regucalcin, which was discovered in 1978 [9], plays a multifunctional role as a suppressor protein in signal transduction in various cell types and plays a cell physiologic role in maintaining cell homeostasis for various stimuli [10-14]. Cytoplasmic regucalcin localizes into the nucleus, and it suppresses nuclear protein kinase and protein phosphatase activities and DNA and RNA synthesis and regulates gene expression for various proteins [15]. Regucalcin has also been shown to suppress protein synthesis and activate proteolysis, suggesting a role as suppressor in protein turnover [11]. Moreover, overexpression of endogenous regucalcin has been demonstrated to suppress cell proliferation and apoptosis induced through multisignaling pathways in various cell types [16,17]. Thus, regucalcin plays a pivotal role in cell regulation.

Moreover, there is growing evidence that regucalcin plays an important role in the regulation of glucose and lipid metabolism. Fasting-induced decrease in the hepatic regucalcin mRNA expression has been shown to restore after re-feeding in rats *in vivo* [18], suggesting that feeding is a physiologic factor in the regulation of the regucalcin gene expression. In addition, oral administration of glucose to fasted rats causes a significant increase in hepatic regucalcin

mRNA expression [18], suggesting an involvement of insulin secreted from pancreatic cells after glucose administration. Moreover, hepatic regucalcin mRNA expression is clearly elevated after a single subcutaneous administration of insulin to fasted rats *in vivo* [18]. In fact, insulin has been demonstrated to directly stimulate regucalcin mRNA and protein expressions in human hepatoma cells (HepG2) *in vitro* [19]. Thus, insulin, which is related to metabolism of blood glucose after feeding, stimulates regucalcin expression in liver cells. In addition, hepatic regucalcin expression has been shown to markedly decrease after a single subcutaneous administration of streptozotocin that induces type 1 diabetes [20]. These findings may support the view that regucalcin may be involved in liver metabolic disorder related to diabetes.

Deficiency of regucalcin has been reported to cause an impairment of glucose tolerance in regucalcin knockout (KO) mice [21]. Regucalcin KO mice causes a significant increase in blood glucose concentration and a decrease in serum insulin levels after glucose administration compared with wild-type mice *in vivo* [22], suggesting that regucalcin participates in the regulation of glucose metabolism related to insulin action. Insulin resistance may be modeled in culture system by using cloned rat hepatoma H4-II-E cells cultured with insulin and TNF- $\alpha$  *in vitro* [22]. This *in vitro* model nicely mimics insulin resistance in human type 2 diabetic mellitus. When H4-II-E cells are cultured in the presence of TNF- $\alpha$  plus insulin *in vitro*, regucalcin is identified as an important protein, which is involved in insulin resistance, by proteome analysis [23]. Thus, regucalcin may be a key molecule that is related to insulin resistance. Moreover, regucalcin has been demonstrated to stimulate glucose utilization and lipid production in H4-II-E cells *in vitro* [24]. Overexpression of endogenous regucalcin is found to stimulate the production of triglyceride and free fatty acid in H4-II-E cells cultured with or without the supplementation of glucose in the absence of insulin [24]. Regucalcin may stimulate lipid production that is linked to glucose metabolism in liver cells *in vitro*. Moreover, the effect of insulin, which enhances medium glucose consumption, triglyceride and free fatty acid productions in liver cells cultured with glucose supplementation, is suppressed by overexpression of regucalcin *in vitro* [25].

Molecular mechanism by which regucalcin regulates glucose

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metabolism related insulin action has been elucidated. Overexpression of regucalcin does not reveal stimulatory effects on the gene expression of enzymes, which are related to glucose and lipid metabolism, including acetyl-CoA carboxylase, HMG-CoA reductase, glucokinase and pyruvate kinase in liver cells after culture with or without glucose supplementation in the presence of insulin [23], although it is possible that regucalcin has a regulatory effect on various enzyme activities, which are related to glucose and lipid metabolism in liver cells. Interestingly, overexpression of regucalcin has been shown to have suppressive effects on the expression of rat insulin receptor (Insr) or phosphatidylinositol 3-kinase (PI3K) mRNAs enhanced after culture with glucose supplementation in the presence of insulin [23,24]. Suppressive effects of regucalcin on the expression of Insr and PI3K mRNAs may play an important role in insulin resistance in liver cells overexpressing endogenous regucalcin. Insulin resistance in the liver is associated with the pathogenesis of nonalcoholic fatty liver disease (NFLD), suggesting an involvement in lipid metabolic disorder.

Hepatocytes, which are obtained from regucalcin KO mice at 12 months of age, have been shown to contain many lipid droplets, abnormally enlarged mitochondria with indistinct cristae, and enlarged lysosomes filled with electron-dense bodies in the electron microscope as compared with that of wild-type mice [25]. Hepatic neutral lipids, total phospholipids, total triglyceride and cholesterol in regucalcin KO mice are found to markedly increase than those from age-matched wild-type mice [25]. Deficiency of regucalcin leads to accumulation of liver lipid components.

Moreover, regucalcin transgenic (TG) rats with overexpression of endogenous regucalcin have been shown to induce a remarkable of bone loss associated with increase in serum triglyceride and high-density lipoprotein (HDL)-cholesterol concentrations at the age of 36 weeks *in vivo* [22]. Serum free fatty acid, triglyceride, cholesterol or HDL-cholesterol concentrations are markedly increased in regucalcin TG male and female rats at 14-50 weeks of age [26]. Thus, hyperlipidemia is uniquely induced in regucalcin TG rats with increasing age. The change in lipid components in the adipose and liver tissues of regucalcin TG rats with increasing age has also been shown *in vivo* [27]. Regucalcin is expressed in the adipose tissues of normal rats [27]. Triglyceride content in the adipose tissues is increased in regucalcin TG rats with aging [27]. Liver triglyceride, total cholesterol, free fatty acid and glycogen contents are decreased in regucalcin TG rats. The expression of regucalcin in the liver tissues is enhanced in regucalcin TG rats [27]. Regucalcin has been shown to have suppressive effects on the activations of glycogen particulate phosphorylase a, cytoplasmic pyruvate kinase, and fructose 1,6-diphosphatase in rat liver [8,13]. Regucalcin may suppress glycogen synthesis in the liver and stimulate glycogenolysis in regucalcin TG rats. As the result, lipid synthesis may be stimulated in the liver tissues of the TG rats *in vivo*. Leptin and adiponectin are adipokines that are involved in lipid metabolism [28]. Leptin mRNA expression in the adipose or liver tissues has been found to decrease in regucalcin TG rats with aging [27]. Adiponectin mRNA expression is not changed in the adipose tissues of the TG rats, while its level is decreased in the liver tissues [27]. These decreases may be partly involved in hyperlipidemia induced in regucalcin TG rats. Thus, regucalcin may play an important role in the disorder of lipid metabolism in the liver.

Hyperlipidemia has been shown to induce in the lipoprotein lipase-deficient mice [28], low-density lipoprotein (LDL) receptor-deficient mice [29], apolipoprotein C3-KO mice [30], apolipoprotein C1 TG mice [31], very LDL lipoprotein receptor KO mice [32], cholesterol

7 alpha-hydroxylase-deficient mice [33], apoE-deficient mice [34], and hepatic myr-Akt overexpressing mice [35]. These animal models for hyperlipidemia are involved in molecules that regulate lipid metabolism. Regucalcin has also been proposed to be a key molecule that regulates lipid metabolism.

As described above, regucalcin plays a physiological role in lipid and glucose metabolism. Regucalcin, which is stimulated by insulin, is identified as a molecule that is related to insulin resistance in liver cells. Deficiency of regucalcin impairs glucose tolerance and induces lipid accumulation in the liver of mice *in vivo*. Overexpression of regucalcin stimulates glycolysis and lipid production in the liver tissues of rats *in vivo*. Disturbance of hepatic regucalcin expression may lead to NFLD. Moreover, hyperlipidemia is induced in regucalcin TG rats *in vivo*. Thus, regucalcin may be a key molecule in lipid metabolic disorder and diabetes. Regucalcin may be a target molecule for therapy of these diseases. Development of further study will be expected.

#### Author Disclosures

The author has no conflicts of interest.

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