Regucalcin, a novel factor implicated in hyperlipidemia

Masayoshi Yamaguchi*
Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, USA

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Abstract
Regucalcin, which was discovered as a calcium-binding protein in 1978, has been demonstrated to play a multifunctional role as a suppressor in signaling transduction in various types of cells and tissues. Regucalcin has been shown to regulate intracellular calcium homeostasis, protein kinases and phosphatases, nitro oxide production, protein synthesis, gene expression, cell proliferation and apoptotic cell death. Regucalcin overexpression and deficiency induces the disorders of bone metabolism, lipid production and glucose metabolism in vivo, indicating an involvement in metabolic disorder. Regucalcin overexpression induces hyperlipidemia implicated in the adipose and liver, and its deficiency caused lipid accumulation in the liver. Regucalcin overexpression stimulates glucose transport and lipid production in liver cells. In addition, regucalcin was found to mediate insulin resistance. Regucalcin regulates the gene expression of insulin receptor and PI3 kinase in liver cells. This review will discuss an involvement of regucalcin in metabolic disorder implicated in lipid metabolism and diabetes.

Keywords: Regucalcin, hyperlipidemia, diabetes, insulin resistance, liver, metabolic disorder

Introduction
Regucalcin, which was discovered as a calcium-binding protein in 1978 [1, 2], plays a multifunctional role as a suppressor in signal transduction in various types of cells and tissues. Regucalcin plays a cell physiologic role in maintaining cell homeostasis for various stimuli [3-9]. The regucalcin gene (rgn) is localized on the X chromosome and is identified in over 15 species consisting of regucalcin family [3]. Regucalcin has been demonstrated to play a potential role in cell regulation of calcium homeostasis, signal transduction, protein synthesis and proteolysis, nuclear gene expression, cell proliferation and apoptosis in various types of cells and tissues including liver, kidney, heart, brain, bone and other tissues [3-9]. Moreover, regucalcin was shown to play a pathophysiologic role in osteoporosis, hyperlipidemia and diabetes. We generated regucalcin transgenic rats that reveal overexpression of endogenous regucalcin, and this animal was found to induce hyperlipidemia associated with osteoporosis [10-12]. Regucalcin was suggested to be a key molecule in lipid metabolic disorder implicated in obesity and diabetes. This review will discuss an involvement of regucalcin in lipid metabolic disorder and diabetes, which is a recent topic.

Regucalcin stimulates adipogenesis
Obesity and diabetes are currently a major health problem worldwide with growing in prevalence. The incidence of metabolic disease, including type 2 diabetes with obesity, is 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. 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 [13]. 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-γ (PPARγ) and runt-related transcription factor 2 (Runx2) [13-15]. Insulin, which is secreted by feeding, stimulates adipogenesis from
bone marrow mesenchymal stem cells. Bone marrow adiposity and mature adipocytes with obesity greatly produces tumor necrosis factor-α (TNF-α), an inflammatory cytokine [16]. This TNF-α may cause insulin resistance that leads to type 2 diabetes. Various hormones and cytokines, which include leptin, adiponectin, insulin, epinephrine, cortisol, glucagon, TNF-α 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.

Regucalcin has been demonstrated to stimulate adipogenesis in mouse bone marrow cell culture *in vitro* [17], suggesting an involvement as a stimulatory factor in adipogenesis. Interestingly, exogenous regucalcin was found to suppress osteoblastogenesis and stimulate adipogenesis in mouse bone marrow culture *in vitro* [17, 18]. Regucalcin may stimulate differentiation from bone marrow mesenchymal stem cells to adipocytes, supporting the view that regucalcin plays a regulatory role in adipogenesis.

Regucalcin transgenic (TG) rats, which overexpress endogenous regucalcin, induced a remarkable bone loss associated with increase in serum triglyceride and high-density lipoprotein (HDL)-cholesterol concentrations at the age of 36 weeks *in vivo* [10-12]. Serum free fatty acid, triglyceride, cholesterol or HDL-cholesterol concentrations were markedly increased in regucalcin TG male and female rats at 14-50 weeks of age [11]. This animal may be a useful tool in the aspects of lipid metabolic disorder with osteoporosis.

Hyperlipidemia has been reported to induce in various animal models; lipoprotein lipase-deficient mice [19], low-density lipoprotein (LDL) receptor-deficient mice [20], apolipoprotein C3-KO mice [28], apolipoprotein C1 TG mice [29], very LDL lipoprotein receptor KO mice [21], cholesterol 7 alpha-hydroxylase-deficient mice [22], apoE-deficient mice [23] and hepatic myr-Akt overexpressing mice [24]. These animal models for hyperlipidemia are based on molecules that are regulated to lipid metabolism. Regucalcin is a novel protein molecule that regulates lipid metabolism [18].

**Involvement of regucalcin in lipid and glucose metabolism**

Regucalcin has been showed to express in the adipose tissues of normal rats [25]. Triglyceride content in the adipose tissues was increased in regucalcin TG rats with aging [25]. Liver triglyceride, total cholesterol, free fatty acid and glycogen contents were decreased in regucalcin TG rats [25]. The expression of regucalcin in the liver tissues was enhanced in regucalcin TG rats [25]. Regucalcin suppressed the activations of glycogen particulate phosphorylase α, cytoplasmic pyruvate kinase, and fructose 1,6-diphosphatase in rat liver [3]. Regucalcin may suppress glycogen synthesis in the liver and stimulate glycolysis 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 involved in lipid metabolism. Leptin mRNA expression in the adipose or liver tissues was found to decrease in regucalcin TG rats with aging [25]. Adiponectin mRNA expression was not changed in the adipose tissues of the TG rats, while its level was decreased in the liver tissues [25]. 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.

Fasting induced a decrease in regucalcin mRNA expression in rat liver *in vivo*, and this decrease was restored after re-feeding in rats *in vivo* [26]. Oral administration of glucose to fasted rats caused a significant increase in hepatic regucalcin mRNA expression [26], suggesting an involvement of insulin secreted from pancreatic cells after glucose administration. Hepatic regucalcin mRNA expression was elevated administration of insulin to fasted rats *in vivo* [26]. Insulin was demonstrated to directly stimulate regucalcin mRNA and protein expressions in human hepatoma cells (HepG2) *in vitro* [27]. Thus, insulin stimulated regucalcin gene expression in liver cells. Hepatic regucalcin expression was markedly decreased after a single subcutaneous administration of streptozotocin that induces type 1 diabetes [28]. These findings support the view that regucalcin gene expression is enhanced by insulin, and that regucalcin may be involved in liver metabolic disorder related to diabetes.

**Involvement of regucalcin in diabetes**

Deficiency of regucalcin has been reported to cause an impairment of glucose tolerance in regucalcin knockout (KO) mice [29, 30]. Regucalcin KO mice caused a significant increase in blood glucose concentration and a decrease in serum insulin levels after glucose administration compared with wild-type mice *in vivo* [29]. Regucalcin deficiency in mice caused an accumulation of neutral lipids and phospholipids in the liver and shortens the life span [29]. Regucalcin was not expressed in hepatic stellate cells (HSCs) of both wild type and regucalcin KO mice [31, 32]. Numerous HSCs were hypertrophic and contained abundant microvesicular lipid droplets in the liver cytoplasm of aged regucalcin KO mice [32]. Deficiency of regucalcin may lead to accumulation of liver lipid components.

Insulin resistance may be modeled in culture system by using cloned rat hepatoma H4-II-E cells cultured with insulin and TNF-α *in vitro* [33]. This *in vitro* model nicely mimicsed insulin resistance in human type 2 diabetic mellitus. When H4-II-E cells were cultured in the presence of TNF-α plus insulin *in vitro*, regucalcin was identified as an important protein, which is involved in insulin resistance, by proteome analysis [33]. Regucalcin may be a key molecule that is related to insulin resistance. Regucalcin, moreover, has been demonstrated to stimulate glucose utilization and lipid production in H4-II-E cells *in vitro* [34]. Overexpression of endogenous regucalcin was 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 [34]. Regucalcin may stimulate lipid production that is linked to glucose metabolism in liver cells in vitro. The effect of insulin, which enhances medium glucose consumption, triglyceride and free fatty acid productions in liver cells cultured with glucose supplementation, was suppressed by overexpression of regucalcin in vitro [35]. Insulin resistance in the liver is associated with the pathogenesis of nonalcoholic fatty liver disease (NAFLD). Patients with NAFLD had a significant lower level of hepatic regucalcin [36]. Hepatic regucalcin levels were decreased in a fibrosis stage-dependent manner and were correlated negatively with the homeostasis model assessment of insulin resistance, the net electronegative charge modified-LDL, and type IV collagen 7S [36]. Whether or not the decrease in hepatic regucalcin in human patients is a result or a cause of cirrhosis remains to be elucidated [36].

Regucalcin has been demonstrated to regulate the genes expression of various proteins that are related to glucose and lipid metabolism in liver cells. Overexpression of regucalcin did not reveal stimulatory effects on the gene expression of enzymes 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 [34]. Overexpression of regucalcin increased the expression of glucose transporter 2 (GLUT 2) mRNA to enhance glucose utilization in the liver cells [34, 35], and it was found to suppress the expression of insulin receptor (Insr) or phosphatidylinositol 3-kinase (PI3K) mRNAs that are an insulin signaling-related protein [34, 35]. Regulatory effects of regucalcin on these gene expressions may play an important role in insulin resistance in liver cells. In addition, regucalcin may suppress signal transduction pathways that are related to insulin action in liver cells.

**Prospects**

Regucalcin may play a physiological role in lipid and glucose metabolism in the adipose and liver as summarized in (Figure 1). Regucalcin was identified to be a molecule related to insulin resistance in liver cells. Deficiency of regucalcin impaires glucose tolerance and induces liver lipid accumulation. Overexpression of regucalcin was found to stimulate hepatic glycolysis and lipid production. Disturbance of hepatic regucalcin gene expression may lead to the disorders of lipid metabolism and diabetes in the liver tissues, resulting in hyperlipidemia. Regucalcin may play a potential pathophyslogic role in lipid metabolic disorder and diabetes. Regucalcin may be a target molecule for therapy of these diseases. Studies on clinical aspect will be expected.

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**Figure 1:** Regucalcin induces hyperlipidemia in vivo. Regucalcin stimulates adipogenesis in the bone marrow, stimulates adipolysis in the adipose, and stimulates lipogenesis in the liver, resulting in hyperlipidemia. Regucalcin enhances glucose transport and inhibits glycolysis in the liver. Moreover, regucalcin reveals insulin resistance by suppressing the gene expression of insulin receptor and PI3 kinase in the liver and stimulating GLUT 2 gene expression.
Author disclosures

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binding protein regucalcin concentration is decreased by streptozotocin-diabetic state and ethanol ingestion in rats. Mol Cell Biochem 168: 67-72