Integrative Molecular Medicine

Review Article ISSN: 2056-6360

Regucalcin, a novel regulatory protein implicated in obesity and diabetes

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Abstract

Regucalcin was initially discovered in 1978 as a unique calcium-binding protein that is a suppressor in intracellular calcium signaling. The regucalcin gene (rgn) is localized on the X chromosome and is identified in over 15 species consisting of regucalcin family. Regucalcin has been demonstrated to play a multifunctional role in cell regulation of calcium homeostasis, signal transduction, nuclear gene expression, cell proliferation and apoptosis in various types of cells and tissues. Moreover, regucalcin plays a pathophysiologic role in hyperlipidemia and diabetes. Liver regucalcin gene expression is stimulated through action of insulin in liver cells and decreased in type I diabetic model animals. Overexpreesion of regucalcin reveals hepatic insulin resistance, decreased liver triglyceride, total cholesterol and glycogen contents in the liver of rats, inducing a hyperlipidemia. Liver leptin and adiponectin mRNA expressions are decreased by overexpression of regucalcin. Deficiency of regucalcin induces an impairment of glucose tolerance and liver lipid accumulation in mice, and it is associated with the development and progression of nonalcoholic fatty liver disease and fibrosis in human patients. Regucalcin may be a key molecule in lipid metabolic disorder implicated in obesity and diabetes.

Introduction

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 [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, winglesstype 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 (PPARγ) 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-α (TNF-α), an inflammatory cytokine [4]. This TNF-α may cause insulin resistance that leads to type 2 diabetes.

Osteoporosis, which bone mass is dramatically reduced after menopause, has also been shown to induce with obesity, diabetes (type I and II) and inflammatory disease [7,8]. Type 1 and type 2 diabetes have been associated with increased fracture risk. Osteoporosis and obesity are now thought to be closely related and to share several features. Osteoporosis and obesity are now thought to be closely related and to share several features [1,2,4]. One of shared features for obesity with osteoporosis is that osteoblasts and adipocytes differentiate from a common precursor cell in the bone marrow mesenchymal stem cells. There is an inverse relationship between differentiation of mesenchymal stem cells to osteoblasts and adipocytes. Secondary causes of osteoporosis including obesity and diabetes are associated with bone marrow adiposity, which greatly produces (TNF-α) [4,5].

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. Moreover, regucalcin, which is a suppressor protein of intracellular signaling systems [9], has been proposed to be a key molecule in obesity and diabetes associated with osteoporosis [10-12]. In addition, regucalcin has been demonstrated to stimulate adipogenesis in mouse bone marrow cell culture *in vitro* [12], suggesting an involvement as a stimulatory factor in adipogenesis. This review will discuss an involvement of regucalcin as a novel protein in obesity and diabetes.

Pivotal role of regucalcin in cell regulation

Regucalcin, which was discovered in 1978 [13,14], 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 [9,15-17]. Cytoplasmic regucalcin localizes into the nucleus, and it suppresses nuclear protein kinase

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Key words: regucalcin, human tumor, alternative spliced valiant, gene expression, carcinogenesis

Received: July 4, 2014; **Accepted:** July 17, 2014; **Published:** July 23, 2014

and protein phosphatase activities and deoxyribonucleic acid and ribonucleic acid 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 [9]. Moreover, overexpression of endogenous regucalcin has been demonstrated to suppress cell proliferation and apoptosis induced through multisignaling pathways in various cell types [16,17]. Regucalcin has been demonstrated to play a pivotal role in cell regulation in various type of cell and tissues including liver, kidney, heart, brain and bone [9,18-20].

Overexpression of regucalcin induces hyperlipidemia associated with osteoporosis

Yamaguchi *et al.* generated regucalcin transgenic rats that reveal overexpression of endogenous regucalcin, and this animal was found to induce hyperlipidemia associated with osteoporosis [21]. Exogenous regucalcin has been shown to stimulate osteoclastic bone resorption and suppress osteoblastic bone formation, thereby decreasing bone mass [19]. Moreover, exogenous regucalcin was found to suppress osteoblastogenesis and stimulate adipogenesis in mouse bone marrow culture *in vitro* [11]. 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 overexpresses 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* [22,23]. 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 [23]. Thus, hyperlipidemia associated with bone loss was found to induce in regucalcin TG rats with increasing age. This animal may be a useful tool in the aspects of lipid metabolic disorder and osteoporosis.

The change in lipid components in the adipose and liver tissues of regucalcin TG rats with increasing age has also been shown *in vivo* [24]. Regucalcin was expressed in the adipose tissues of normal rats [24]. Triglyceride content in the adipose tissues was increased in regucalcin TG rats with aging [24]. This may be an implication with adipogenesis that is enhanced by regucalcin [11]. Liver triglyceride, total cholesterol, free fatty acid and glycogen contents were decreased in regucalcin TG rats [24]. The expression of regucalcin in the liver tissues was enhanced in regucalcin TG rats [24]. Regucalcin suppressed the activations of glycogen particulate phosphorylase a, cytoplasmic pyruvate kinase, and fructose 1,6-diphosphatase in rat liver [9]. 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 adiponectine are adipokines that are involved in lipid metabolism [25]. Leptin mRNA expression in the adipose or liver tissues was found to decrease in regucalcin TG rats with aging [24]. Adiponectin mRNA expression was not changed in the adipose tissues of the TG rats, while its level was decreased in the liver tissues [24]. These decreases may be partly involved in hyperlipidemia induced in regucalcin TG rats. Regucalcin may play an important role in the disorder of lipid metabolism in the liver. The role of regucalcin in the regulation of lipid metabolism and implication with obesity and diabetes is summarized in Figure 1.

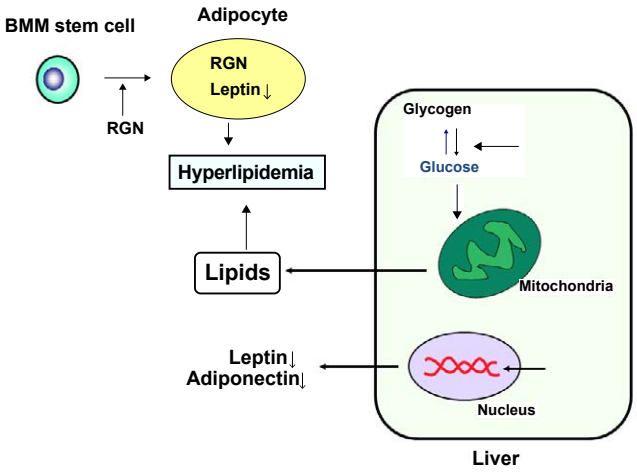

Figure 1: Role of regucalcin (RGN) in the regulation of lipid metabolism and implication with obesity and diabetes. RGN stimulates differentiation from bone marrow mesenchymal (BMM) stem cells to adipocytes, and it enhances adipogenesis and enhances triglyceride in the adipocytes. RGN suppresses leptin expression in adipocytes that regulate feeding. RGN stimulates glycolysis and production of in the liver cells, and it suppress adiponectin and leptin mRNA expression in the liver cells.

Hyperlipidemia has been reported to induce in various animal models; lipoprotein lipase-deficient mice [26], low-density lipoprotein (LDL) receptor-deficient mice [27], apolipoprotein C3-KO mice [28], apolipoprotein C1 TG mice [29], very LDL lipoprotein receptor KO mice [30], cholesterol 7 alpha-hydroxylase-deficient mice [31], apoE-deficient mice [32] and hepatic myr-Akt overexpressing mice [33]. 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 [12].

Role of regucalcin in hepatic lipid metabolism

Regucalcin plays an important role in the regulation of glucose and lipid metabolism [12]. 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* [34], suggesting that feeding is a physiologic factor in the regulation of the regucalcin gene expression. In addition, oral administration of glucose to fasted rats caused a significant increase in hepatic regucalcin mRNA expression [34], suggesting an involvement of insulin secreted from pancreatic cells after glucose administration. Moreover, hepatic regucalcin mRNA expression was clearly elevated after a single subcutaneous administration of insulin to fasted rats *in vivo* [34]. In fact, insulin was demonstrated to directly stimulate regucalcin mRNA and protein expressions in human hepatoma cells (HepG2) *in vitro* [35]. Insulin, which is related to metabolism of blood glucose after feeding, 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 [36]. These findings may support the view that regucalcin gene expression is enhanced by insulin, and 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 [37,38]. 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* [38], suggesting that regucalcin participates in the regulation of glucose metabolism

related to insulin action. Regucalcin deficiency in mice caused an accumulation of neutral lipids and phospholipids in the liver and shortens the life span [37]. Hepatocytes, which were obtained from regucalcin KO mice at 12 months of age, was 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 [37]. Hepatic neutral lipids, total phospholipids, total triglyceride and cholesterol in regucalcin KO mice were found to markedly increase than those from age-matched wild-type mice [37].

Regucalcin was not expressed in hepatic stellate cells (HSCs) of both wild type and regucalcin KO mice [39,40]. Peroxisome proliferator-activated receptor-gamma (PPAR-γ) was up-regulated in the liver of regucalcin KO mice [39]. Numerous HSCs was hypertrophic and contained abundant microvesicular lipid droplets in the liver cytoplasm of aged regucalcin KO mice [39,40]. The expression of PPAR-γ, which is a protein related to lipid metabolism and HSC quiescence, was also found to increase in hypertrophic HSCs of regucalcin KO mice [39]. Deficiency of regucalcin leads to accumulation of liver lipid components.

Regucalcin is involved in insulin resistance

Insulin resistance may be modeled in culture system using cloned rat hepatoma H4-II-E cells cultured with insulin and TNF-α *in vitro* [41]. 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 [41]. 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* [42]. 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 [42]. 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* [43].

Insulin resistance in the liver is associated with the pathogenesis of nonalcoholic fatty liver disease (NAFLD). Change in hepatic regucalcin levels may be associated with the development and progression of NAFLD [44]. Patients with NAFLD had a significant lower level of hepatic regucalcin [44]. Hepatic regucalcin levels 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 [44]. Both serum large very LDL and very small LDL levels were elevated in patients with NAFLD [44]. Whether or not the decrease in hepatic regucalcin in human patients is a result or a cause of cirrhosis remains to be elucidated, however [44]. Insulin resistance in the liver is associated with the pathogenesis of NFLD, suggesting an involvement in lipid metabolic disorder.

Molecular mechanism by which regucalcin regulates glucose metabolism related insulin action has been elucidated. Overexpression of regucalcin did 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 [42]. Overexpression of regucalcin increased the expression of glucose transporter 2 (GLUT 2) mRNA to enhance glucose utilization in the liver cells [42,43], 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 [42,43]. 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. Moreover, regucalcin may suppress signal transduction pathways related to insulin action in liver cells.

Prospect

Regucalcin may play a physiological role in lipid and glucose metabolism in the adipocytes and liver those are implicated in obesity and diabetes. Regucalcin, which its gene expression is enhanced by insulin, is identified as a molecule related to insulin resistance in liver cells. Deficiency of regucalcin impairs glucose tolerance and induces liver lipid accumulation. Overexpression of regucalcin stimulates hepatic glycolysis and lipid production. Disturbance of hepatic regucalcin gene expression may leads to disorders of lipid metabolism and insulin resistance in liver tissues, inducing a hyperlipidemia. Regucalcin may play a pathophysiologic role as a regulatory protein implicated in obesity and diabetes. Regucalcin may be a target molecule for therapy of these diseases.

Acknowledgements

The author was supported by the Foundation for Biomedical Research on Regucalcin.

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