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Regucalcin and metabolic disorders: osteoporosis and hyperlipidemia are induced in regucalcin transgenic rats

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Abstract Regucalcin transgenic (TG) rat has been generated to determine the role in metabolic disorders. Regucalcin homozygote male and female rats induce a prominent increase in regucalcin protein in the various tissues. Bone loss has been found to induce in regucalcin TG rats with growing (5 weeks old) and aging (50 weeks old). Osteoclastogenesis has been shown to stimulate in culture with the bone marrow cells obtained from regucalcin TG rats. Exogenous regucalcin stimulates osteoclastogenesis in mouse marrow culture in vitro. Regucalcin has a suppressive effect on the differentiation and mineralization in osteoblastic MC3T3-E1 cells in vitro. The mechanism by which regucalcin TG rat induces bone loss may result from the enhancement of osteoclastic bone resorption and the suppression of osteoblastic bone formation. Moreover, regucalcin TG rat has been found to induce hyperlipidemia with increasing age (14–50 weeks); serum triglyceride, high-density lipoprotein (HDL)-cholesterol, free fatty acid, albumin and calcium concentrations are markedly increased in regucalcin TG male and female rats with increasing age. The decrease in lipid and glycogen contents in liver tissues is induced in regucalcin TG rats. The gene expression of leptin and adiponectin is suppressed in the TG rats. Overexpression of regucalcin has been shown to enhance glucose utilization and lipid production in the cloned rat hepatoma H4-II-E cells in vitro, and insulin resistance is seen in the cells. The expression of glucose transporter 2 mRNA is increased in the transfectants, while it has been shown to suppress insulin receptor and phosphatidylinositol 3-kinase mRNA expressions that are involved in insulin signaling. This review proposes that regucalcin relates in osteoporosis and hyperlipidemia, and that the regucalcin TG rat model may be useful in determining the pathophysiologic state and the development of therapeutic tool for osteoporosis and hyperlipidemia.

Keywords Regucalcin · RGN · Bone · Osteoporosis · Lipid · Lipidemia · Insulin resistance · Transgenic rat

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Introduction

Calcium ion (Ca^{2+}) plays an important role in the regulation of many cell functions. The Ca^{2+} effect on cells is amplified by Ca^{2+}-dependent protein kinases that relate to a signal transduction due to hormonal stimulation [1–3]. Regucalcin (RGN) was found in the year of 1978 as a novel Ca^{2+}-binding protein that differs from calmodulin and other Ca^{2+}-related proteins as it does not contain an EF-hand motif of Ca^{2+}-binding domain [4–7]. The name regucalcin was proposed for this Ca^{2+}-binding protein that may regulate the effect of Ca^{2+} on cell functions [8–12]. After that, the identical protein to regucalcin has also been reported as senescence marker protein-30 (SMP30) [13, 14]. There are growing evidences that regucalcin plays a multifunctional role as a regulatory protein in Ca^{2+}-dependent and -independent signaling processes in many cell types (reviewed in References [15–20]).

Regucalcin and its gene have been identified in 16 species consisting of regucalcin family [7, 21, 22]. Comparison of the nucleotide sequences of regucalcin from vertebrate

species is highly conserved in their coding region, and they conserve throughout evolution [21, 22]. The gene is localized on the chromosome X in rat and human [23, 24]. The organization of rat regucalcin gene consists of seven exons and six introns, and several consensus regulatory elements exist upstream of the 5′-flanking region [25]. AP-1 [26], NFI-A1 [27, 28], RGPR-p117 [29–31], and β-catenin [32] have been found to be a transcription factor for the enhancement of regucalcin gene promoter activity. The transcription activity of the regucalcin gene is enhanced by various intracellular signaling factors. This enhancement is partly caused by the phosphorylation of nuclear proteins that mediate through various protein kinases related to intracellular signaling [33–38].

Regucalcin greatly expresses in the liver and kidney cortex [39–41]. Regucalcin is also found in other tissues and many cells including brain, heart, bone, breast, lung, and prostate [42–46], suggesting that the protein has a role in the regulation of their tissue functions. The expression of regucalcin mRNA is regulated by the administration of various factors including calcium [41], insulin [47], 17β-estradiol and androgen [45, 46, 48] in the tissues of rats in vivo.

Regucalcin has a multifunctional role in maintaining intracellular Ca^{2+} homeostasis by activating Ca^{2+} pump enzymes; suppressing Ca^{2+} signaling from the cytoplasm to the nucleus in the proliferative cells; inhibiting protein kinases, protein phosphatases, protein synthesis, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis in the cytoplasm and nucleus of cells and suppressing apoptotic cell death induced by various signaling factors (reviewed in References [15–20]). From these findings, regucalcin has been proposed to play a pivotal role in maintaining cell homeostasis and function as the regulatory protein of intracellular signaling system [20].

The physiologic role of regucalcin in vivo has been poorly understood, however. The deficiency of regucalcin (SMP30) induces a decrease in L-ascorbic acid (vitamin C) in mice [49]. This may not be significant, because vitamin C is not synthesized in human.

We studied a role of endogenous regucalcin in vivo using a transgenic (TG) rat model [50]. The great expression of endogenous regucalcin is demonstrated in the various tissues of regucalcin TG rats [50]. The regulatory effect of endogenous regucalcin on various enzyme activities that are seen by using normal rat tissues in vitro [51] has also been demonstrated in regucalcin TG rats in vivo [50]. Regucalcin TG rat was a good model to determine the role of endogenous regucalcin with overexpression in body function. We found that osteoporosis and hyperlipidemia are induced in regucalcin TG rats, indicating a pathophysiologic role of regucalcin in metabolic disorder [44, 52].

This review has been written to outline the recent advances that have been made concerning the mechanism of action of regucalcin in the osteoporosis and hyperlipidemia that are induced in regucalcin TG rats in vivo and in vitro.

Generation of regucalcin TG rats

Regucalcin TG rat was generated by pronuclear injection of the transgene with a cDNA construct encoding rat regucalcin [50]. TG founders were fertile, transmitted the transgene at the expected frequency, and bred to homozygote. Western analysis of the cytosol prepared from the tissue from TG female rats (5 weeks old) showed a remarkable expression of regucalcin (33 kDa) protein in the liver, kidney cortex, heart, lung, stomach, brain, spleen, muscle, colon, duodenum, and others [50]. The expression of regucalcin in TG male rats was seen in the liver, kidney cortex, heart, and lung. In wild type (male and female rats), regucalcin mainly was present in the liver and kidney cortex [50]. Sexual differences were seen in the tissue expression of regucalcin in the TG rats [50].

Regucalcin TG rat is registered and preserved in The National BioResource Project for the Rat in Japan (NBRP Rat No:0166, Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Kyoto, Japan), and it can get commercially from Japan SLC (Hamamatsu, Japan; http://www.jslc.co.jp/).

Regucalcin has been shown to have a suppressive effect on protein phosphatase activity in the cytosol of liver and kidney cortex from wild-type rats [51]. Dephosphorylation of many phosphorylated proteins is regulated by protein phosphatase in cells [52]. The effect of regucalcin in suppressing protein phosphatase activity was enhanced in the cytosol of kidney cortex of TG male and female rats as compared with those of wild-type rats [50]. Such an effect was associated with the enhancement of regucalcin expression in the TG rats. The decrease in protein phosphatase activity in the TG rats was completely abolished in the presence of anti-regucalcin monoclonal antibody in the enzyme reaction mixture in vitro [50]. These observations demonstrate that the endogenous regucalcin has a suppressive effect on protein phosphatase activity in vivo.

Regucalcin has been shown to increase Ca^{2+}-ATPase activity in the microsomes (sarcoplasmic reticulum) of rat heart muscle [43]. The Ca^{2+} current is one of the most important components in cardiac excitation–contraction coupling. This coupling mechanism is based on the regulation of intracellular Ca^{2+} concentration by Ca^{2+} pump in the sarcoplasmic reticulum of heart muscle. Regucalcin TG male and female rats caused a remarkable increase in regucalcin expression in the cytosol and microsomes of heart muscle [50]. The microsomal Ca^{2+}-ATPase activity was raised in the heart muscle of the TG male and female rats [50]. This may support the view that endogenous

regucalcin has a role as an activator for Ca^{2+}-ATPase in the microsomes of rat heart muscle in vivo. The increase in heart muscle microsomal Ca^{2+}-ATPase activity that was seen in the TG female rats was completely abolished after the addition of anti-regucalcin monoclonal antibody [50]. This indicates that the change in the enzyme's activity in the tissues of regucalcin TG rats is related to an increase in endogenous regucalcin.

The body weight of regucalcin TG female rats was significantly lowered as compared with that of wild-type rats [50]. Serum inorganic phosphorus concentration was increased in TG male and female rats, while serum glucose, triglyceride, free cholesterol, albumin and urea nitrogen concentrations were not altered in the TG rats (5 weeks old) [50]. These observations suggest that a specific metabolic disorder, which relates to regulation of serum inorganic phosphorus concentration, is induced in regucalcin TG weanling rats.

Thus, regucalcin TG rat has been shown to be a useful model to define a role of endogenous regucalcin in various tissues in vivo.

Bone loss is induced in regucalcin TG rats

The role of regucalcin in bone homeostasis has not been determined so far. Regucalcin mRNA expression and its protein levels were found in the femoral-diaphyseal and -metaphyseal tissues of normal (wild type) rats [44]. These levels were increased in the femoral-metaphyseal tissues of regucalcin TG male rats and in the diaphyseal and metaphyseal tissues of the TG female rats [44].

The morphologic change in the femoral-diaphyseal and -metaphyseal tissues of regucalcin TG rats was demonstrated by using a peripheral quantitative computed tomography (pQCT); morphologic change was great in the femoral tissues of female rats as compared with that of male rats [44]. Regucalcin TG female rats induced a remarkable decrease in mineral content, mineral density and polar strength strain index in the femoral-diaphyseal and -metaphyseal tissues [44], as is shown in Fig. 1. A significant decrease in cortical thickness was seen in the femoral-diaphyseal tissues from regucalcin TG female rats. Calcium contents in the femoral-diaphyseal and -metaphyseal tissues were decreased in regucalcin TG male and female rats; a remarkable decrease was seen in the female rats [44]. Regucalcin may play an important role in the regulation of bone metabolism.

Thus, bone morphologic change was found to induce in regucalcin TG rats and the generation of bone morphologic change in regucalcin TG rats was remarkable in the female rats [44]. This finding suggests that regucalcin plays a role in the stimulation of bone resorption and that it has a suppressive effect on bone formation.

Bone biochemical markers were clearly altered in regucalcin TG rats [44]. The femoral-metaphyseal alkaline phosphatase activity was significantly lowered in regucalcin TG female rats [44]. The enzyme activity was not changed in the femoral-diaphyseal tissues of the TG female rats. In the diaphyseal tissue of male rats, the enzyme activity was decreased in the TG rats. Alkaline phosphatase is a marker enzyme of osteoblasts [54]. The observation that the enzyme activity is decreased in the femoral tissues of regucalcin TG rats suggests a decrease in osteoblastic bone formation.

DNA content in the femoral-diaphyseal and -metaphyseal tissues was markedly decreased in regucalcin TG female rats [44]. In the TG male rats, the femoral-metaphyseal DNA content was reduced. DNA content in the

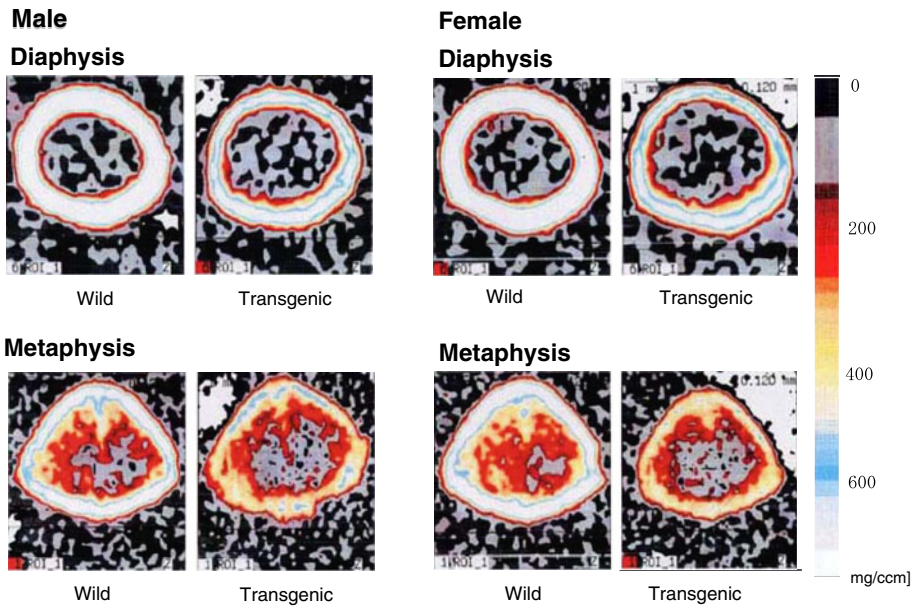

Fig. 1 Bone morphologic change in the femoral-diaphyseal and -metaphyseal tissues of normal (wild type) and regucalcin transgenic (TG) rats. Periheral quantitative computed tomography (pQCT) analysis was carried out on the femur of wild-type or TG rats (5 weeks old)

bone tissues may be an index of bone growth and the number of bone cells in femoral tissue [55]. The decrease in bone cells (including osteoblastic cells) and chondrocytes may be induced in regucalcin TG rats. These cells may greatly be present in the femoral-metaphyseal tissues. Presumably, bone formation and bone growth are retarded in the femoral-metaphyseal tissues of regucalcin TG rats.

The observation with alteration of biochemical components further supports the view that bone loss is induced in the femoral tissue of regucalcin TG rats which are estimated with bone morphologic index [44]. The cellular mechanism by which the overexpression of regucalcin induces bone loss remains to be elucidated, however. This may give a novel aspect in the regulatory mechanism of bone homeostasis. Regucalcin may involve in the promotion of osteoclastogenesis from bone marrow cells and the regulation of cell functions of osteoclasts and osteoblasts.

Regucalcin TG female rat induced a remarkable decrease in bone mass as compared with that of the TG male rats [44]. Bone loss with increasing age is well known to lead osteoporosis [56, 57]. Ovarian hormone deficiency with aging induces bone loss [57]. Post-menopausal osteoporosis may result from estrogen deficiency. Whether regucalcin TG female rat is estrogen deficient, is unknown. However, the bone loss in regucalcin TG female rats was seen with independent of age. The regucalcin TG rat model may be useful in determining a novel pathogenic mechanism in the progression of osteoporosis.

Osteoclastic bone resorption is induced in regucalcin TG rats in vivo

The finding that bone loss is induced in regucalcin TG rats [44] suggests that regucalcin involves in the stimulation of osteoclastic bone resorption. Osteoclasts are differentiated from bone marrow cells, and their formation is stimulated by various bone-resorbing factors [58]. The expression of regucalcin was demonstrated in the marrow cells of normal (wild type) rats using Western blot analysis [59]. Regucalcin levels were significantly increased in the marrow cells of regucalcin TG male and female rats with increasing age (5–36 weeks old).

When the marrow cells obtained from wild-type rats or regucalcin TG rats (36 weeks old) were cultured for 7 days without addition of bone-resorbing factors in vitro, the number of tartrate-resistant acid phosphatase (TRACP) -positive multinucleated cells (MNCs) was increased in the marrow culture of regucalcin TG rats [59]. This increase was a remarkable in TG female rats as compared with the male rats. TRACP is a marker enzyme of osteoclasts [58]. Bone loss, which was induced in regucalcin TG rats, was remarkable in female rats as compared with that of male rats [44]. Presumably, the mechanism by which regucalcin TG rat induces bone loss is partly involved in the enhancement of osteoclast-like cell formation in the TG rats.

The effect of parathyroid hormone [human PTH (1–34)] or 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] in stimulating TRACP-positive MNCs formation was found to enhance in female regucalcin TG rats [58]. Such an effect was not observed in the male TG rats. This finding may partly support the observation that bone loss is greatly induced in regucalcin TG female rats.

Whether regucalcin expresses in mononuclear pre-osteoclasts and osteoclasts is unknown at present. It is speculated, however, that regucalcin may act on the process of differentiation of bone marrow cells. It appears that intracellular regucalcin plays a regulatory role in osteoclastic cell formation from marrow cells. Regucalcin plays a role as a regulatory protein in the process of intracellular signaling in the liver, kidney cortex, and brain of wild-type rats [20]. It is possible that intracellular regucalcin enhances the effect of hormone or cytokine on osteoclastogenesis in bone marrow cells.

Bone loss was seen from 5 weeks old of regucalcin TG rats [44]. The decrease in calcium content in the femoral-diaphyseal and -metaphyseal tissues of regucalcin TG rats was also observed in male and female TG rats with increasing age (5 and 36 weeks old). Femoral-metaphyseal DNA content was reduced in regucalcin TG male and female rats (36 weeks old). Thus, bone loss was induced in regucalcin TG rats with weanling and aging.

Interestingly, serum inorganic phosphorus, triglyceride, HDL-cholesterol and albumin concentrations were also found to elevate in regucalcin TG female rats of 36 weeks old [59]. A significant increase in serum inorganic phosphorus concentration was seen in regucalcin TG male and female rats of 5 weeks old [44]. Serum lipid components were not changed in regucalcin TG rats of 5 weeks old. However, serum triglyceride and HDL-cholesterol concentrations were raised in the TG rats with increasing age, suggesting that the TG rats with aging induces the disorder of lipid metabolism which associates with bone loss. Triglyceride, HDL-cholesterol and albumin in the serum are produced in the liver. Liver metabolism may be attenuated in regucalcin TG rats with increasing age.

Osteoclastic bone resorption in regucalcin TG rats was enhanced with increasing age [60]. Femoral-diaphyseal and -metaphyseal tissues were obtained from normal (wild type) or regucalcin TG rats of 5, 14, 25, or 50 weeks old. Calcium content in the femoral-diaphyseal and -metaphyseal tissues was decreased in regucalcin TG male and female rats of 5, 14, 25, or 50 weeks old as compared with the value obtained from wild-type rats of each age [60]. When the marrow cells obtained from wild-type or regucalcin TG rats were cultured for 7 days, the number of

TRACP-positive MNCs was found to increase after culture of marrow cells obtained from regucalcin TG male and female rats of 5, 14, 25, or 50 weeks old [60]. The effect of PTH or $1,25(OH)_2D_3$ in stimulating TRACP-positive MNCs formation was enhanced in regucalcin TG male and female rats with 14 or 25 weeks old [60]. Thus, osteoclastic bone resorption was found to enhance with increasing age in regucalcin TG male and female rats [60].

The formation of osteoclast-like cells was shown to stimulate in the culture of bone marrow cells obtained from regucalcin TG rats without addition of bone-resorbing factors [58]. Moreover, the increase in osteoclast-like cell formation in regucalcin TG rats was found to enhance with increasing age [60]. However, the decrease in femoral-diaphyseal and -metaphyseal calcium content in regucalcin TG rats was not greatly reduced with increasing age [60]. Endogenous suppressive factors seem to be secreted to inhibit the function of osteoclasts in regucalcin TG rats.

Regucalcin was expressed in the bone marrow cells of both wild-type and regucalcin TG rats [60]. Osteoclastogenesis was stimulated in the culture of bone marrow cells from regucalcin TG rats [60]. It is speculated that regucalcin is released from bone marrow cells of regucalcin TG rats, and that its extracellular protein has a stimulatory effect on the formation of osteoclasts in bone marrow cells. It is possible that regucalcin, as a cytokine, has a direct stimulatory effect on osteoclast-like cell formation in bone culture system. This remains to be elucidated, however.

It is well established that receptor activator of NF-kB ligand (RANKL) plays a key role in the development of osteoclasts from pre-osteoclasts [59, 61]. RANKL binds to RANK (receptor for RANKL) in pre-osteoclasts, and stimulates differentiation to osteoclasts. It cannot exclude the possibility; however, that endogenous regucalcin in pre-osteoclastic cells may partly enhance the stimulatory effect of RANKL on osteoclast-like cell formation in regucalcin TG rats.

Regucalcin directly stimulates osteoclastogenesis in vitro

The direct stimulatory effect of exogenous regucalcin on bone resorption and osteoclastogenesis in bone marrow culture in vitro has been investigated [62]. When rat femoral-diaphyseal or -metaphyseal tissues were cultured for 48 h in the presence of regucalcin (10^{-10}–10^{-8} M), the diaphyseal or metaphyseal calcium content was decreased in vitro [62]. The consumption of glucose and the production of lactic acid in the diaphyseal or metaphyseal tissues were increased after culture with regucalcin [62]. Such an effect of regucalcin was also seen in the presence of PTH, which is a physiologic important bone-resorbing hormone. These observations demonstrate that regucalcin directly stimulates bone resorption in rat femoral tissues in vitro [62].

Receptor activator of NF-kB ligand (RANKL) plays a pivotal role in the generation of osteoclasts from pre-osteoclast that their differentiation is enhanced in the presence of macrophage colony-stimulating factor (M-CSF) [59, 61]. RANKL is secreted from osteoblasts. PTH, $1,25(OH)_2D_3$, or prostaglandin E_2, which are bone-resorbing factors, stimulates the expression of RANKL in osteoblasts. RANKL binds to RANK (receptor for RANKL) in pre-osteoclasts and stimulates differentiation to osteoclasts.

Regucalcin was found to stimulate osteoclast-like cell formation, when mouse marrow cells were cultured for 7 days in the presence of both RANKL and M-CSF [62]. Such an effect was not observed after the addition of regucalcin at the later stage of culture in the presence of both RANKL and M-CSF [62]. However, the effect of regucalcin in stimulating osteoclast-like cell formation was seen after its addition at the later stage of culture in the absence of both RANKL and M-CSF [62]. It is speculated that regucalcin acts the process of differentiation from pre-osteoclasts to osteoclasts independent on M-CSF and RANKL. Whether regucalcin has an osteoclastogenic effect in spleenocytes remains to be elucidated, however.

The effect of regucalcin in stimulating osteoclast-like cell formation was inhibited in the presence of cycloheximide, an inhibitor of protein synthesis, or 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), an inhibitor in transcription activity, suggesting that the effect of regucalcin is resulted from newly synthesized protein components [62]. The effect of regucalcin in stimulating osteoclastic cell formation may not involve in the enhancement of RANKL expression or the suppression of osteoprotegerin (OPG) expression from osteoblastic cells in bone marrow culture system. It is speculated that regucalcin stimulates osteoclastogenesis independently on the mechanism through RANKL/OPG. Exogenous regucalcin may directly bind to pre-osteoclasts and may stimulate differentiation to osteoclasts.

The effect of various bone-resorbing factors [PTH, $1,25(OH)_2D_3$, prostaglandin E_2, or tumor necrosis factor-α (TNF-α)] in stimulating osteoclast-like cell formation in mouse marrow culture was found to enhance in the presence of regucalcin [62]. Interestingly, the effect of TNF-α on osteoclast-like cell formation was markedly enhanced in the presence of regucalcin [62]. TNF-α is an autocrine factor in pre-osteoclasts, which promotes their cell differentiation, and it mediates RANKL's induction of osteoclastogenesis [63]. Regucalcin may modulate the effect of TNF-α on osteoclastogenesis.

The effect of regucalcin in stimulating osteoclast-like cell formation in mouse marrow culture was inhibited in

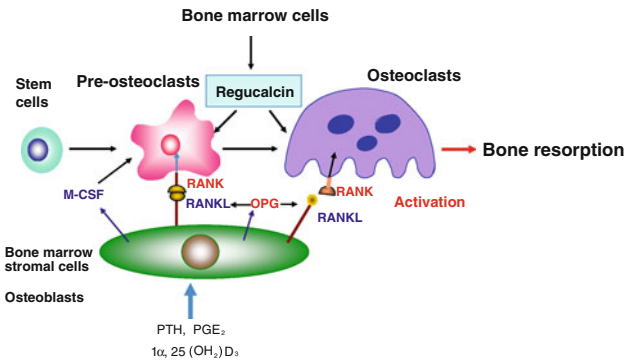

Fig. 2 Regucalcin stimulates osteoclastogenesis in the bone marrow cells of regucalcin TG rats. Regucalcin may be secreted from bone marrow cells. Regucalcin may directly bind to pre-osteoclasts and stimulate differentiation to osteoclasts. The effect of regucalcin in stimulating osteoclastic cell formation may not be involved in the enhancement of RANKL expression or the suppression of OPG expression from osteoblastic cells in bone marrow culture system

the presence of various anti-bone-resorbing factors including calcitonin and 17β-estradiol [62]. It is well known that calcitonin or 17β-estradiol inhibits osteoclastic bone resorption.

As mentioned above, regucalcin can directly stimulate osteoclastic bone resorption in vitro. The mechanism by which regucalcin stimulates osteoclastic bone resorption is summarized in Fig. 2. Regucalcin has a role as a novel bone-resorbing factor.

Regucalcin suppresses osteoblastic bone formation in vitro

As mentioned above, osteoclastic bone resorption has been shown to induce in regucalcin TG rats in vivo [44, 58, 60] and regucalcin has been demonstrated to stimulate directly osteoclastogenesis in marrow cultures in vitro [62]. Moreover, regucalcin has been found to have a suppressive effect on osteoblastic bone formation in vitro [64].

Osteoblastic MC3T3-E1 cells with subconfluent monolayers were cultured in a medium containing regucalcin (10^{-10}–10^{-8} M) without fetal bovine serum (FBS). The expression of regucalcin mRNA and its protein was demonstrated by using reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis [64].

Regucalcin did not have the effect on cell proliferation and apoptosis in osteoblastic cells with culture of short term [64]. The targeted disruption of transcription factors runt-related transcription factor 2 (Runx2) has been shown to result in a complete lack of bone formation owing to maturational arrest of osteoblasts [65]. The expression of Runx2 mRNA was found to suppress in osteoblastic cells with subconfluency cultured with short term in the presence of regucalcin [64]. The levels of α1 (I) collagen and glyceroaldehyde-3-phosphate dehydrogenase (G3PDH) mRNAs in osteoblastic cells were not changed after culture with regucalcin [64]. Regucalcin-induced suppression of Runx2 mRNA expression may lead to the deterioration of cell differentiation and mineralization in osteoblastic cells.

Insulin-like growth factor-I (IGF-I) plays a role in the stimulation of bone formation in osteoblastic lineage cells [55]. The expression of IGF-I mRNA was found to suppress in osteoblastic cells after culture with regucalcin addition [64]. This suppression may partly have an effect in inducing the deterioration of osteoblastic bone formation. Meanwhile, the expression of transforming growth factor-β1 (TGF-β1) mRNA in osteoblastic cells was enhanced after culture with regucalcin addition [64]. This stimulation was seen with 1-day culture. TGF-β1 is a potent regulator of osteoblast differentiation of osteoblastic cells [66]. Regucalcin-enhanced TGF-β1 mRNA expression may be partly involved in the suppression of differentiation of osteoblastic cells.

Alkaline phosphatase activity was markedly decreased in osteoblastic cells cultured with short term in the presence of regucalcin (10^{-10}–10^{-8} M) [64]. The enzyme participates in bone mineralization process [54]. The decrease in alkaline phosphatase activity in osteoblastic cells after culture with regucalcin addition may influence on osteoblastic mineralization. The suppressive effect with regucalcin addition on the enzyme activity may be mediated through intracellular signaling factors that are generated after stimulation with regucalcin in osteoblastic cells.

Moreover, the prolonged culture with regucalcin induced a remarkable suppression of mineralization in osteoblastic cells [64]. When osteoblastic cells with subconfluency were cultured with addition of regucalcin (10^{-10} or 10^{-9} M) for 3, 9, or 18 days, the results with Alizarin red staining showed that mineralization was suppressed after culture with regucalcin for 3, 9, or 18 days [64]. This finding demonstrates that regucalcin has a suppressive effect on cell differentiation and mineralization in osteoblastic cells [64].

From these observations, it is speculated that extracellular regucalcin that is released from osteoblastic cells binds to the plasma membranes of the cells, and that the protein transmits a signal into the cells to regulate gene expression and mineralization.

The effect of endogenous regucalcin in osteoblastic cells was examined. When regucalcin was added into the enzyme reaction mixture containing the lysate of osteoblastic cells that were cultured without regucalcin addition, alkaline phosphatase activity was decreased [64]. Intracellular regucalcin may have a regulatory effect on alkaline phosphatase activity in osteoblastic cells.

Ca^{2+}/calmodulin-dependent nitric oxide (NO) synthase activity in the cell lysate was decreased after addition of

regucalcin (10^{-10}–10^{-8} M) into the reaction mixture [64]. The presence of anti-regucalcin monoclonal antibody (5 or 10 ng/ml) in the enzyme reaction mixture caused a significant increase in NO synthase activity in the cell lysate in the presence or absence of Ca^{2+}/calmodulin, suggesting a regulatory role of endogenous regucalcin [64]. NO synthase produces NO in many cell types, and NO plays a role in the regulation of cell function [67].

Thus, intracellular regucalcin has been demonstrated to have a suppressive effect on the activities of alkaline phosphatase and NO synthase in osteoblastic cells [64]. Regucalcin has also been demonstrated to play a role as a regulatory protein of intracellular signaling in many cell types [20]. Likewise, regucalcin, which is expressed in osteoblastic cells, may play a regulatory role in the regulation of osteoblastic cell function.

The effect of exogenous regucalcin is mediated through signaling factors in osteoblastic cells

Iodinated regucalcin has been shown to bind to the plasma membranes isolated from rat liver in vitro [68]. It appears that exogenous regucalcin binds to the plasma membranes of osteoblastic cells and that the protein transmits signal(s) into the cells [69]. It is speculated that specific binding sites for regucalcin are localized on osteoblastic cells.

Whether the effect of exogenous regucalcin in osteoblastic cells is mediated through intracellular signaling factor was examined by using pharmacologic tool [68]. Osteoblastic MC3T3-E1 cells with subconfluent monolayers were cultured for 24–72 h in a medium containing regucalcin (10^{-10} or 10^{-9} M) without FBS. The addition of regucalcin did not have an effect on cell number. Culture with regucalcin for 24 h caused a significant decrease in protein and DNA contents in osteoblastic cells [68]. The effect of regucalcin in decreasing protein contents in osteoblastic cells was inhibited in the presence of various kinase inhibitors including protein kinase C, Ca^{2+}/calmodulin-dependent protein kinase, phosphatidylinositol 3-kinase, and mitogenic activated protein (MAP) kinase [68]. This suggests that the effect of regucalcin in decreasing cellular protein content is partly mediated through various protein kinases that are involved in intracellular signaling process.

Culture with regucalcin caused a decrease in DNA content in osteoblastic cells in the presence of various kinase inhibitors [68]. The effect of regucalcin in decreasing cellular DNA content may be involved in other signaling mechanisms that differ from its action on cellular protein content. It is assumed that the effect of exogenous regucalcin is mediated through several signaling pathways in osteoblastic cells.

The effect of regucalcin in decreasing cellular protein content was also seen in the presence of IGF-I that can stimulate cell proliferation [70]. However, the regucalcin addition-induced decrease in cellular DNA content was not observed in the presence of IGF-I [68]. Presumably, the effect of regucalcin is transmitted independent on the intracellular signaling process of IGF-I action.

The addition of regucalcin did not have an effect on protein and DNA contents in the cells cultured with cycloheximide, an inhibitor of protein synthesis, or DRB, an inhibitor of transcription activity, although each inhibitor caused a significant decrease in those contents [68]. These observations may support the view that exogenous regucalcin regulates gene expression that is mediated through signaling factors [68].

Culture with regucalcin was found to suppress the mRNA expressions of Runx2, a transcription factor [65, 71], and alkaline phosphatase, a key enzyme of mineralization [54, 72], in osteoblastic cells [68]. The effect of regucalcin may be transmitted to transcription process in the nucleus of osteoblastic cells. At present, the signaling factors that mediate the action of regucalcin are unknown. However, various protein kinases may be partly related to the intracellular signaling of regucalcin action in osteoblastic cells.

The cellular mechanism by which regucalcin suppresses osteoblastic bone formation is summarized in Fig. 3.

Hormonal regulation of regucalcin mRNA expression in osteoblastic cells

The expression of regucalcin mRNA in osteoblastic MC3T3-E1 cells in vitro was shown to regulate by various hormones and cytokines [73]. Cells with subconfluency were cultured for 24 or 48 h in a medium containing either vehicle or various hormones without FBS. PTH, $1,25(OH)_2D_3$, or TNF-α stimulates osteoclastic bone resorption [74]. PTH, IGF-I, or 17β-estradiol has an anabolic effect on osteoblastic cell function [75, 76]. The receptors of these hormones and cytokines are expressed in osteoblastic MC3T3-E1 cells.

Culture with PTH, IGF-I, or 17β-estradiol enhanced the expression of regucalcin mRNA in osteoblastic cells [73]. Meanwhile, the expression of regucalcin mRNA was suppressed after culture with $1,25(OH)_2D_3$ or TNF-α [73]. TNF-α had a potent suppressive effect on regucalcin mRNA expression, suggesting that the cytokine has a role as the negative regulator in regucalcin mRNA expression. The effect of TNF-α is mediated through NF-κB signaling that is involved in TNF receptor-associated factor (TRAF6) in osteoblastic cells [74]. The suppressive effect of TNF-α on regucalcin mRNA expression in osteoblastic cells may be mediated through NF-κB signaling. This suggests the

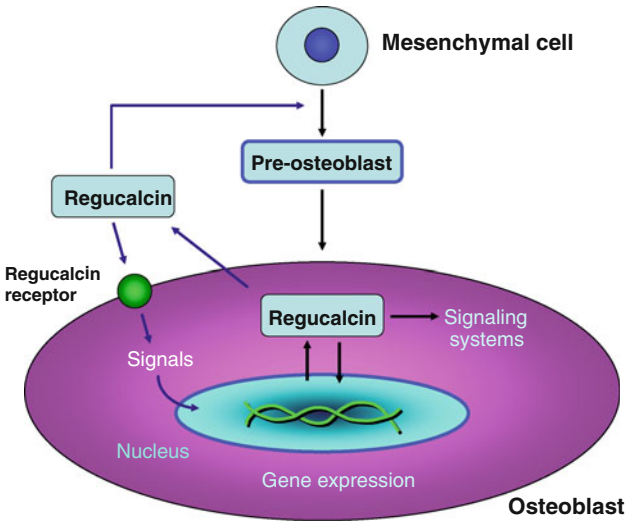

Fig. 3 Regucalcin suppresses differentiation and mineralization in osteoblastic cells. Regucalcin may be secreted from osteoblastic cells in regucalcin TG rats, and it suppresses differentiation from mesenchymal cells to pre-osteoblasts, and it also binds the receptors of regucalcin in osteoblastic cells. The effect of regucalcin is transmitted through signaling system that is involved in various protein kinases. Moreover, intracellular Regucalcin may regulate signaling system-related enzyme activation and gene expression in the nucleus of osteoblastic cells

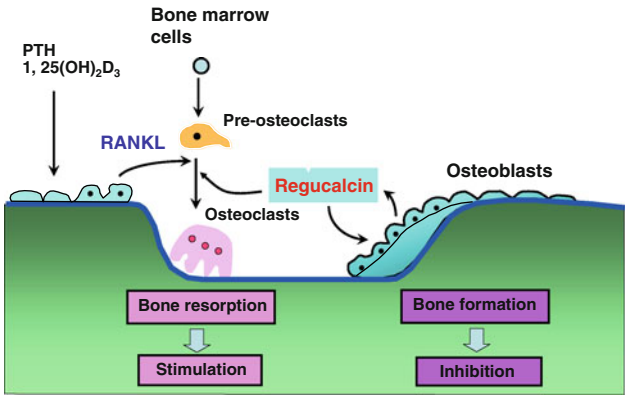

Fig. 4 Regucalcin plays an important role in the regulation of bone homeostasis due to stimulating osteoclastic bone resorption and inhibiting osteoblastic bone formation. This may induce bone loss in regucalcin TG rats

existence of NF-kB binding sites in the promoter region of regucalcin gene in osteoblastic cells.

The effect of PTH or IGF-I in increasing regucalcin mRNA expression in osteoblastic cells was completely suppressed after culture with staurosporine, an inhibitor of protein kinase C, or PD98059, an inhibitor of MAP kinase [73]. The expression of regucalcin mRNA in osteoblastic cells may be enhanced through intracellular signaling factors that are involved in various protein kinases.

17β-estradiol may have a stimulatory effect on regucalcin mRNA mediated through the steroid receptors in the cells. In addition, the expression of regucalcin mRNA in osteoblastic cells was decreased after a longer culture with $1,25(OH)_2D_3$, suggesting that its expression is suppressed through the steroid receptors.

Regucalcin had a suppressive effect on cell function that is involved in differentiation and mineralization of osteoblastic cells [64]. Moreover, the expression of regucalcin mRNA is regulated by various hormones and cytokines that involve in the regulation of osteoblastic cell function. Regucalcin may have a part in the regulation of the effects of hormone and cytokine in osteoblastic cells.

In conclusion, bone loss was induced in the femoral tissues of regucalcin TG rats [44]. Regucalcin was expressed in rat bone marrow cells. Osteoclastic cell formation from bone marrow cells was enhanced in regucalcin TG rats [58]. Osteoclastic bone resorption was stimulated in regucalcin TG rats with increasing age [60]. Exogenous regucalcin stimulated osteoclastogenesis in bone marrow cell [62]. Regucalcin suppressed osteoblastic differentiation and mineralization in vitro [64]. Regucalcin is a novel protein that plays a role in the regulation of bone homeostasis due to stimulating osteoclastic bone resorption and inhibiting osteoblastic bone formation, as is shown in Fig. 4.

Hyperlipidemia is induced in regucalcin TG rats

The endogenous regucalcin in the TG rats had a regulatory effect on hepatic nuclear protein phosphatase activity [77], renal cortex cytosolic NO synthase activity [78], heart sarcoplasmic reticulum Ca^{2+}-ATPase and cytosolic NO synthase [43, 79], and brain cytosolic NO synthase and protein tyrosine phosphatase activities [80] in vivo. These observations coincided with their regulatory effects of exogenous regucalcin in vitro using the subcellular fraction of various tissues obtained from normal rats. Thus, the physiologic role of regucalcin in the regulation of cellular function has been demonstrated in vivo using regucalcin TG rats. In the aspect of pathophysiologic state, regucalcin TG rat was found to induce osteoporosis [58, 60], which is a phenotype in the TG rats.

Interestingly, hyperlipidemia was also induced in regucalcin TG rats with increasing age (14, 25, 36, or 50 weeks) [50, 53]. Serum triglyceride, HDL-cholesterol, and free fatty acid concentrations were markedly increased in regucalcin TG male and female rats at 25, 36, or 50 weeks of age [53]. The change in serum HDL-cholesterol concentration in regucalcin TG rats with increasing age is shown in Fig. 5.

Serum albumin concentration was also elevated in regucalcin TG female rats at 25, 36, or 50 weeks of age [53]. Moreover, serum calcium concentration was found to

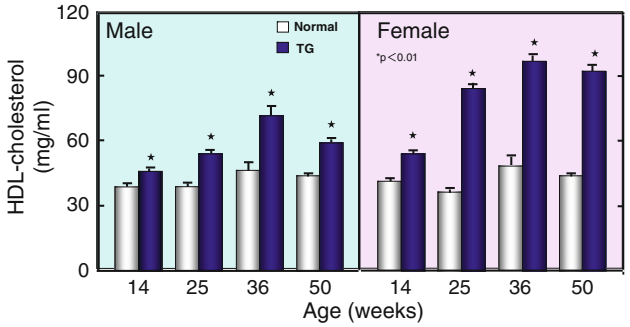

Fig. 5 Change in serum HDL-cholesterol concentration in regucalcin TG rats with increasing age. Serum HDL-cholesterol concentration siginificantly ($P < 0.01$) increased in the TG rats as compared with that of wild-type rats

elevate in regucalcin TG male and female rats at 50 weeks of age [53]. Serum zinc, glucose, or urine nitrogen concentrations were not altered in TG male and female rats [53]. Hyperlipidemia was observed from the early stage with increasing age in regucalcin TG rats. Hyperlipidemia that is induced in regucalcin TG rats with increasing age may be unique [53]. Regucalcin TG rat may be useful as an animal model for hyperlipidemia associated with osteoporosis.

Hyperlipidemia has been shown to induce in the lipoprotein lipase-deficient mice [81], low-density lipoprotein (LDL) receptor-deficient mice [82], apolipoprotein C3-knockout mice [83], apolipoprotein C1 transgenic mice [84], very low-density lipoprotein receptor knockout mice [85], cholesterol 7 alpha-hydroxylase-deficient mice [86], apoE-deficient mice [87], and hepatic myr-Akt overexpressing mice [88]. These animal models for hyperlipidemia are involved in molecules that regulate lipid metabolism. Regucalcin may be a novel molecule that participates in lipid metabolism.

Regucalcin is greatly expressed in the liver of rats [39, 40]. Regucalcin has a suppressive effect on the activations of particulate glycogen phosphorylase *a* [10], liver cytosolic pyruvate kinase [9], and fructose 1,6-diphosphatase [8] by Ca^{2+}/calmodulin in the liver of rats, indicating that regucalcin plays a role in the regulation of liver glucose metabolism. Regucalcin may have a regulatory effect on glucose metabolism in liver. It is assumed that hyperlipidemia is related to production of lipids in the liver of regucalcin TG rats. The role of regucalcin in the regulation of hepatic lipid metabolism has been poorly understood, however.

Regucalcin TG rat also caused a significant increase in serum albumin concentration with increasing age [53]. Overexpression of regucalcin may stimulate production of albumin from the liver of the TG rats. The pathophysiologic significance which regucalcin TG rat causes an increase in serum albumin concentration remains to be elucidated.

Serum calcium concentration was significantly raised in regucalcin TG rats at 50 weeks of age [53]. Hypercalcemia may be related to bone loss that is induced in regucalcin TG rats. A significant increase in serum inorganic phosphorus concentration was seen in regucalcin TG rats of 5, 14, 25, or 35 weeks old, but it was not seen in the TG rats of 50 weeks old [53]. Bone loss was induced in regucalcin TG rats with those ages. Hyperphosphatemia and hypercalcemia may also be involved in bone loss in the TG rats. Thus, regucalcin TG rats in female rather than male have been shown to induce various metabolic disorders, suggesting a pathophysiologic importance. Regucalcin TG rat may be useful as animal model in the study of experimental metabolic diseases.

Lipid metabolism in regucalcin TG rats

Whether lipid components in the adipose and liver tissues are changed in regucalcin TG rats in vivo was examined [89]. Regucalcin TG female rats were used at 7 or 50 weeks old. Serum triglyceride or HDL-cholesterol concentrations were significantly increased in regucalcin TG rats of 7 weeks old as compared with those in normal rats of the same age [89]. Serum triglyceride, total cholesterol, HDL-cholesterol and free fatty acid concentrations were increased in regucalcin TG rats of 50 weeks old [89].

The disorder of lipid metabolism in the adipose and liver tissues, moreover, was found to induce in regucalcin TG rats with increasing age [89]. Triglyceride content in the adipose tissues was increased in regucalcin TG rats of 50 weeks old, while the free fatty acid content was not changed. Liver triglyceride, total cholesterol and free fatty acid contents were decreased in regucalcin TG rats of 50 weeks old [89]. Liver glycogen content was markedly decreased in regucalcin TG rats of 7 or 50 weeks old [89]. Hyperlipidemia that is induced in regucalcin TG rats may be involved in the change in lipid components in the adipose and liver tissues of regucalcin TG rats.

Regucalcin mRNA and its protein were expressed in the adipose tissues of normal rats. However, regucalcin expression in the adipose tissues was not increased in the TG rats [89]. Whether regucalcin in the adipose tissues stimulates the release of lipid components into the serum in regucalcin TG rats is unknown. Triglyceride content in the adipose tissues was increased in regucalcin TG rats [89]. Presumably, the increase in serum lipid components may partly result from the release of lipids from the adipose tissues.

Serum triglyceride concentration was increased in regucalcin TG rats of 7 weeks old as compared with that of normal rats [89]. Triglyceride contents in the adipose and liver tissues were not changed in regucalcin TG rats of

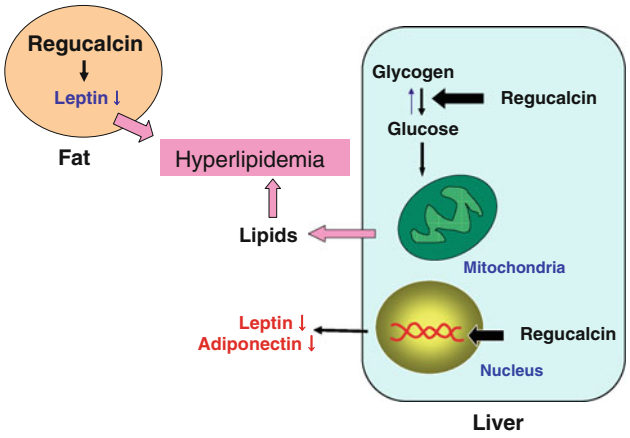

Fig. 6 The disorder of lipid metabolism in the adipose and liver tissues is induced in regucalcin TG rats with increasing age. Triglyceride content in the adipose tissues is increased in regucalcin TG rats, while triglyceride, total cholesterol and free fatty acid contents in the liver tissues are decreased in the TG rats. Hyperlipidemia that is induced in regucalcin TG rat may be resulted from the increase in lipid components in the liver tissues and the suppression of leptin or adiponectine mRNA expression in adipose or liver tissues of regucalcin TG rats

7 weeks old. Meanwhile, glycogen content in the liver tissues was decreased in regucalcin TG rats of 7 weeks old, suggesting that hepatic glycogen synthesis is suppressed in regucalcin TG rats [89]. The expression of regucalcin in the liver tissue was enhanced in regucalcin TG rats [44]. Regucalcin has been shown to have a suppressive effect on glycogen phosphorylase *a* activity in the glycogen particulate prepared from rat liver [10]. It is speculated that regucalcin suppresses glycogen synthesis in the liver and that the protein stimulates glycolysis in regucalcin TG rats. As the result, lipid synthesis may be stimulated in the liver tissues of regucalcin TG rats. Overexpression of regucalcin has been shown to enhance glucose utilization and lipid production in the cloned rat hepatoma H4-II-E cells in vitro [90]. In addition, regucalcin has been found to involve in insulin resistance in H4-II-E cells in vitro [90–92].

The expression of leptin mRNA in the adipose and liver tissues was found to decrease in regucalcin TG rats of 50 weeks old [89]. Adiponectin mRNA levels were not changed in the adipose tissues of regucalcin TG rats of 50 weeks old, while its expression was decreased in the liver tissues [89]. Leptin and adiponectin are adipokines that are involved in lipid metabolism [93, 94]. Leptin or adiponectin regulates hyperlipidemia [95, 96]. These decreases may be partly involved in the hyperlipidemia that is induced in regucalcin TG rats.

As mentioned above, hyperlipidemia that is induced in regucalcin TG rat may be resulted from the increase in hepatic lipid production and the suppression of leptin or adiponectin mRNA expression in the adipose and liver tissues in the TG rats, as is summarized in Fig. 6.

Overexpresion of regucalcin enhances hepatic lipid production in vitro

Hyperlipidemia was induced in regucalcin TG rats [53], suggesting that lipid components are produced in the liver cells. To determine this, whether overexpression of regucalcin stimulates the production of lipids was examined by using the cloned rat hepatoma H4-II-E cells in vitro [90]. Interestingly, insulin resistance was found to induce in the regucalcin-overexpressed rat hepatoma H4-II-E cells.

The cloned rat hepatoma cells (wild type) and stable regucalcin/pCXN2-transfected cells (transfectant) were cultured for 72 h in a medium containing 10% FBS to obtain subconfluent monolayers. Cells with subconfluency were cultured for 24 or 72 h in a medium containing either vehicle or insulin (10^{-8} or 10^{-7} M) with or without supplementation of glucose (10, 25, or 50 mg/ml of medium) in the absence of insulin.

The productions of triglyceride and free fatty acid were significantly increased in the transfectants that were cultured without insulin and glucose supplementation as compared with that of wild-type cells [90]. The supplementation of glucose caused a remarkable increase in medium glucose consumption, triglyceride and free fatty acid productions in the transfectants cultured without insulin [90]. These findings suggest that overexpression of regucalcin stimulates productions of triglyceride and free fatty acid in the H4-II-E cells. It is speculated that overexpression of regucalcin stimulates glucose utilization in the H4-II-E cells, and that it promotes the production of triglyceride and free fatty acid which are linked to glucose metabolism in the cells in vitro [90].

Insulin had a stimulatory effect on the consumption of medium glucose and the productions of triglyceride and free fatty acid in H4-II-E cells (wild type) cultured with or without the supplementation of glucose [90]. These increases were found to prevent in the transfectants. Overexpression of regucalcin may have a suppressive effect on insulin-stimulated glucose consumption in the H4-II-E cells, indicating insulin resistance.

The gene expression of acetyl-CoA carboxylase, HMG-CoA reductase, glucokinase, and pyruvate kinase, which are rate-limiting enzymes related to glucose and lipid metabolism, in the H4-II-E cells (wild type) and the transfectants was not changed after culture with or without glucose supplementation in the presence of insulin [90]. The expression of glucose transporter 2 (GLUT 2) mRNA was found to increase in the transfectants as compared with that of wild-type cells cultured without insulin and glucose supplementation [90]. Overexpression of regucalcin did not have a stimulatory effect on the gene expression of enzymes, which are related to glucose and lipid metabolisms, in the H4-II-E cells. It is possible, however, that

endogenous regucalcin has an activatory effect on various enzyme activities, which are related to glucose and lipid metabolism, in H4-II-E cells. In addition, it is assumed that regucalcin has a regulatory role in signal transduction of insulin in H4-II-E cells. Regucalcin has been shown to have suppressive effects on protein tyrosine kinase and insulin action in liver cells [97].

Other studies show that insulin resistance may be modeled in H4-II-E liver cells in tissue culture with the use of the cytokine TNF-α and insulin [92]. From the proteome analysis of H4-II-E cells exposed to insulin and TNF-α, it has been proposed that regucalcin has a role associated with proteins that are involved in insulin resistance [98]. However, whether regucalcin can regulate the effect of insulin in liver cells was unknown. This was confirmed by our observations that overexpression of regucalcin induces insulin resistance in the H4-II-E cells [90, 91].

Both insulin resistance and increased oxidative stress in the liver are associated with the pathogenesis of nonalcoholic fatty liver disease. Recently, the pathogenesis of nonalcoholic fatty liver disease in patients has been reported to induce a decrease in hepatic regucalcin (SMP30) with a fibrosis stage-dependent manner and an increase in serum LDL, although it is not known whether decreased hepatic regucalcin (SMP30) is a result or a cause of cirrhosis [99]. The impairment of the early phase of insulin secretion in islets due to dysfunction of the distal portion of the secretion pathway has also been reported to underlie glucose intolerance in regucalcin (SMP30) knockout mice and decreased regucalcin (SMP30) may contribute to the worsening of glucose tolerance that occurs in normal aging [100]. These observations may be related to our early findings that overexpression of regucalcin enhances utilization of glucose and production of lipid components in the liver H4-II-E cells in vitro [90]. Presumably, regucalcin plays a part in the regulation of glucose and lipid metabolism.

Overexpression of regucalcin suppresses the gene expression of insulin signaling-related proteins in the hepatoma cells

As mentioned above, overexpression of regucalcin was shown to enhance glucose utilization and lipid production in the cloned rat hepatoma H4-II-E cells in vitro, and it induces insulin resistance [91]. The effect of regucalcin on the gene expression of insulin signaling-related proteins was examined by using RT-PCR with specific primers in the cloned rat hepatoma cells overexpressing regucalcin in vitro [91].

The hepatoma cells (wild type) and stable regucalcin/pCXN2-transfected cells (transfectants) were cultured for 72 h in a medium containing 10% FBS to obtain subconfluent monolayers. Cells with subconfluency were cultured for 24, 48, or 72 h in a medium containing either vehicle or insulin (10^{-9}–10^{-7} M) with or without supplementation of glucose (10, 25, or 50 mg/ml of medium). The expression of rat insulin receptor (Insr), phosphatidylinositol 3-kinase (PI3K), glucose transporter 2 (GLUT 2), or G3PDH mRNAs was determined. Overexpression of regucalcin was found to enhance GLUT 2 mRNA in the H4-II-E cells, although it failed to have a significant effect on the expression of Insr, PI3K, or G3PDH mRNAs in the cells cultured in the absence of insulin [91]. The expression of GLUT 2 mRNA in the transfectants may be partly involved in the enhancement of glucose utilization in the H4-II-E cells overexpressing regucalcin [91].

Overexpression of regucalcin, moreover, was found to have a suppressive effect on the expression of Insr and PI3K mRNAs that are enhanced after culture with insulin, glucose supplementation, or insulin plus glucose addition [91]. These observations suggest that overexpression of regucalcin suppresses gene expression of insulin signaling-related proteins, indicating that overexpression of regucalcin induces insulin resistance in the cloned rat hepatoma H4-II-E cells in vitro.

The mRNA expression of acetyl-CoA carboxylase, HMG-CoA reductase, glucokinase, and pyruvate kinase, which are related to glucose and lipid metabolism in liver cells, in the cloned rat hepatoma H4-II-E cells was not changed after culture with or without glucose supplementation in the presence of insulin [91]. The suppressive effect of regucalcin on Insr and PI3K mRNA expression may be important in inducing insulin resistance in the cloned rat hepatoma H4-II-E cells overexpressing regucalcin.

In conclusion, regucalcin TG rat induced a remarkable increase in lipid components in the serum [53]. The decrease in lipid component and glycogen in the liver tissues was found in regucalcin TG rats in vivo [89]. Moreover, overexpression of regucalcin enhanced glucose utilization and lipid production in the cloned rat hepatoma H4-II-E cell model in vitro and it suppressed the effect of insulin in the cells. Overexpression of regucalcin in the H4-II-E cells suppressed the gene expression of Insr and PI3K mRNAs that are related to insulin signaling in liver cells, inducing insulin resistance [91]. The regulatory effect of regucalcin in liver tissues, which is related to insulin signaling, may be important in hyperlipidemia induced in regucalcin TG rats, as is summarized in Fig. 7.

Thus, the disorder of lipid metabolism has been demonstrated to induce in regucalcin TG rats, indicating its usefulness as an animal model of hyperlipidemia.

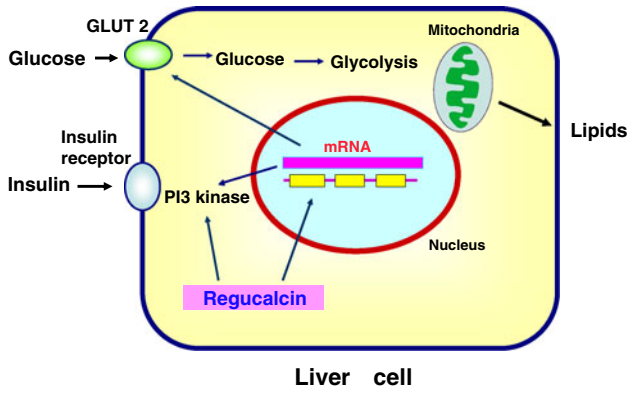

Fig. 7 Overexpression of regucalcin stimulates glucose utilization and lipid production in the cloned rat hepatoma H4-II-E cells. Regucalcin increases GLUT 2 mRNA expression to enhance glucose utilization in the cells. Regucalcin suppresses the gene expression of Insr or PI3K that is an insulin signaling-related protein, which is enhanced after culture with insulin and/or glucose supplementation. Overexpression of regucalcin induces insulin resistance in the cells

Prospects

Regucalcin has been demonstrated to play a multifunctional role as a regulatory protein in Ca^{2+} signaling and other signaling processes in many cell types (reviewed in References [15–20]); maintaining intracellular Ca^{2+} homeostasis; inhibiting protein kinases and protein phosphatases; suppressing protein synthesis and DNA and RNA synthesis; inhibiting cell proliferation; and suppressing apoptotic cell death. From these findings, regucalcin has been proposed to play a pivotal role in maintaining cell homeostasis and function as the regulatory protein of intracellular signaling system [20].

The cellular regulation of regucalcin that is mentioned above has also been demonstrated in regucalcin TG rats in vivo, supporting the view that regucalcin plays a physiologic role in cellular regulation.

Overexpression of regucalcin has been found to induce osteoporosis and hyperlipidemia in the TG rats with weanling and aging, indicating that regucalcin plays a physiologic role in the regulation of bone homeostasis and lipid metabolism. The exploration of the mechanism by which regucalcin TG rat induces bone loss and hyperlipidemia proposes the information that regucalcin is a novel protein which contributes in the regulation of bone and lipid metabolisms, suggesting a physiologic role of regucalcin.

Moreover, hyperphosphatemia, hypercalcemia, and hyperalbuminemia associated with osteoporosis and hyperlipidemia have been found to induce in regucalcin TG rats. Presumably, regucalcin plays a role in the regulation of various functions in living body. Further studies may be able to find other novel roles of regucalcin using the TG rats.

Regucalcin TG rat may be a good animal model in the exploration of mechanism of metabolic disorders and the development of their therapeutic tools.

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