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BREAKTHROUGHS AND VIEWS

The Role of Regucalcin in Nuclear Regulation of Regenerating Liver

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Regucalcin was discovered in 1978 as a Ca^{2+}-binding protein that does not contain EF-hand motif of Ca^{2+}-binding domain [Yamaguchi, M., and Yamamoto T., *Chem. Pharm. Bull.* 26, 1915–1918, 1978]. The name regucalcin was proposed for this Ca^{2+}-binding protein, which can regulate liver cell functions related to Ca^{2+}. Regucalcin has been demonstrated to play a multifunctional role in liver and kidney cells, for which regucalcin mRNA expression and its protein content are pronounced. Hepatic regucalcin mRNA expression has been shown to be mediated through signaling pathway of Ca^{2+}/calmodulin-dependent protein kinase, protein kinase C, and tyrosine kinase. AP-1- and NF-1-like factors can bind to the promotor region of the rat regucalcin gene to mediate the Ca^{2+} response for transcriptional activation. Growing evidence supports the view, moreover, that regucalcin plays an important role in the regulation of Ca^{2+} signaling from the cytoplasm to nuclei in the proliferative cells of regenerating rat liver. Also, regucalcin has been demonstrated to be transported to liver nucleus, and it can inhibit nuclear protein kinase, protein phosphatase, and DNA and RNA synthesis in regenerating liver. Regucalcin plays a physiologic role in the control for overexpression of proliferative cells. Regucalcin has been proposed to be an important regulatory protein in nuclear signaling system.

Key Words: **regucalcin; Ca^{2+} signaling; Ca^{2+}-binding protein; SMP-30; gene expression; regenerating liver.**

Calcium ion (Ca^{2+}) plays an important role in the regulation of many cell functions. A role as second messengers of Ca^{2+} in cells for hormonal stimulation comes into notice. Ca^{2+} signal is partly transmitted to intracellular responses, which are mediated through a family of Ca^{2+}-binding protein. The Ca^{2+} effect is amplified by calmodulin and protein kinase C (1–6). It has been found that a novel Ca^{2+}-binding protein regucalcin, which does not contain EF-hand motif as a Ca^{2+}-binding domain and differs from calmodulin and other Ca^{2+}-related protein, is present in the hepatic cytoplasm of rats (7–10). The name regucalcin is proposed for this Ca^{2+}-binding protein, which can regulate Ca^{2+} effect on liver cell functions (11–19).

In recent years, regucalcin has been demonstrated to play a multi-functional role in liver, kidney and brain neuronal cells (see References 20–22 for review). Regucalcin plays a role in the maintenance of intracellular Ca^{2+} homeostasis due to activating Ca^{2+} pump enzymes in the plasma membrane (basolateral membrane) (23–27), microsomes (28–30) and mitochondria (31–34) of liver and renal cortex cells. Also, regucalcin has an inhibitory effect on the activation of Ca^{2+}/calmodulin-dependent protein kinase (35–39), protein kinase C (36, 37, 40, 41), and protein phosphatases (42–46) which plays an important role in intracellular signaling system. Moreover, regucalcin has an inhibitory effect on protein synthesis (47, 48) and an activatory action on cysteinyl protease including calpaine (49–51). Regucalcin may play an important role as a regulatory protein in the cellular functions of liver, kidney cortex and brain neurons.

Growing evidence supports the views, furthermore, that regucalcin plays an important role in the regulation of Ca^{2+} signaling from the cytoplasm to nucleus in the proliferative cells of regenerating rat liver. Interestingly, regucalcin has been demonstrated to regulate nuclear function in liver cells; it can inhibit Ca^{2+}-activated DNA fragmentation (19), DNA and RNA synthesis (52, 53), protein kinases (37) and protein phosphatases (44, 45) activities in the nuclei of regenerating rat liver. Regucalcin may play a physiologic role in the control for overexpression of proliferative cells.

This review introduces a regulatory mechanism of the regucalcin gene expression which is mediated through Ca^{2+} signaling and a role of regucalcin in the regulation of nuclear function in the proliferative cells of regenerating rat liver. Regucalcin is proposed to play an important role in the regulation of signal transduc-

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tion from the cytoplasm to nucleus in proliferative liver cells.

REGULATION OF REGUCALCIN GENE EXPRESSION BY SIGNALING FACTORS

The rat regucalcin gene is localized on the proximal end of the rat chromosome Xq 11.1-12 (54), and the gene is demonstrated in human, mouse, cow, monkey, rat, rabbit, and chicken but not yeast (55). The organization of the rat regucalcin gene seems to be about six introns (56). We have cloned cDNA for regucalcin from human (57), rat (10, 58), mouse (59), rabbit (60), bovine (60), chicken (60) and toad livers (60). Comparison analysis reveals that the nucleotide sequences of regucalcin from seven vertebrate species were highly conserved in their coding region. The overall regucalcin proteins in the these species consisted of 299 amino acids, and they had 69.9–91.3% identity (60). A great conservation of the regucalcin genes throughout evolution is demonstrated. Regucalcin may be a protein which is highly differentiated. Meanwhile, it has been reported that a cDNA clone encoding rat senescence marker protein-30 (SMP-30) (61), which had not entirely clarified the function of the protein at this time point, is identical to regucalcin, supporting the substantial evidence for a Ca^{2+}-binding protein regucalcin which is isolated from rat liver in 1978 years (7, 8, 10, 58).

Regucalcin mRNA expression (58) and its protein content (62) is pronounced in liver and renal cortex. The expression of regucalcin mRNA (58, 63) and its protein (64) in the liver and renal cortex of rats is clearly stimulated by an increase in the cellular Ca^{2+} levels following an oral administration of calcium chloride to rats *in vivo.* Hepatic regucalcin mRNA expression is also stimulated by a single subcutaneous administration of calcitonin (65), insulin (66) and estrogen (67). Hepatic regucalcin mRNA expression is increased with fetal development, and its expression is stimulated by the intake of dietary calcium *in vivo* (68). Also, aging had been shown to decrease liver regucalcin mRNA expression (58, 69), although such a decrease is not seen in rat kidney (9). Regucalcin mRNA expression in the liver and kidney cortex of rats is partly mediated through Ca^{2+}/calmodulin *in vivo* (63, 70). Moreover, regucalcin mRNA expression has been demonstrated to be mediated through signaling pathway of Ca^{2+}-dependent protein kinases and tyrosine kinase in the cloned rat hepatoma (H4-II-E) cells cultured with 10% fetal bovine serum (71, 72).

There are many regulatory elements (Hox, AP-1, AP-2, and NF-1) in the 5′-flanking region of rat regucalcin gene (56, 73–76). The promoter activity of the rat regucalcin gene is enhanced by treatment with Bay K 8644, dibutyryl cyclic AMP, phorbol esters, insulin, and dexamethasone. Using gel mobility shift assays, it is found that nuclear proteins from rat liver cells and rat hepatoma H4-II-E cells specifically bind to the 5′-flanking region of the rat regucalcin gene (73–76). Bay K 8644, dibutyryl cyclic AMP, phorbol esters and insulin can stimulate the binding of nuclear factors to the 5′-flanking region of the rat regucalcin gene in H4-II-E cells. These factors-inducible nuclear proteins are related to promotor activity of the regucalcin gene (76).

As a specific transcriptional nuclear protein, AP-1 factor has been found to bind to the regucalcin gene in the liver and renal cortex of calcium-administered rats (73, 76). Ca^{2+}-induced binding of the AP-1 factor to the regucalcin gene is completely inhibited by simultaneous administration of trifluoperazine, an antagonist of calmodulin, suggesting that the activation of nuclear AP-1 protein is partly mediated through a Ca^{2+}/calmodulin-dependent pathway (73). AP-1 factor is complex of c-fos/c-jun which is phosphorylated by protein kinases. Ca^{2+}signaling system may be an important pathway in the stimulation of regucalcin mRNA expression.

A nuclear factor I (NF1) consensus motif TTGGC $(N)_6$ CC is present between position −523 and −506 in the 5′-flanking region of the rat regucalcin gene (76). This oligonucleotide competes with the probe for the binding of the nuclear proteins from rat liver and H4-II-E cells. The mutation of TTGGC in the consensus sequence causes an inhibition of the binding of nuclear factors. The presence of Bay K 8644, insulin, and phorbol esters stimulates the binding of the nuclear factors to the TTGGC region of the rat regucalcin gene in H4-II-E cells. The specific mutation introduced in this region, which is ligated to a luciferase reporter gene, reduces significantly the effects of Bay K 8644, insulin, and phorbol esters in stimulating the regucalcin gene transcriptional activity in H4-II-E cells. The specific nuclear factor may bind to the NF1-like sequence, which can stimulate the transcriptional activity, in the promoter region of regucalcin gene in liver cells. The expression of regucalcin gene through this cis-acting element in the NF1-like sequence may be stimulated by signaling factors which are related to Ca^{2+}-dependent protein kinases.

ROLE OF REGUCALCIN IN THE NUCLEAR REGULATION OF REGENERATING LIVER

Growing evidence supports the view that regucalcin plays an important role in the regulation of nuclear function of normal rat liver and regenerating rat liver. Interestingly, regucalcin has been demonstrated to be transported to isolated liver nucleus (44). Regucalcin localization in rat liver nuclei is increased in the proliferative cells of regenerating liver after partial hepatectomy (44). Additionally, the expression of regucalcin

mRNA is enhanced in regenerating rat liver (77). The reduced liver weight by partial hepatectomy (about 70%) is restored at 3 days after surgery. Liver regucalcin mRNA levels are clearly increased 1–5 days after partial hepatectomy, although the increase is not seen 12 h after the surgery (77). Hepatic regucalcin mRNA levels are remarkable at 24 h after partial hepatectomy, indicating that the enhancement of the mRNA expression occurs at the S phase of cell cycle. This finding suggest a correlation with regucalcin in the proliferation of liver cells. This enhancement may be mediated through Ca^{2+}/calmodulin-dependent protein kinase, protein kinase C, and tyrosine kinase (72, 77). In fact, Ca^{2+}/calmodulin-dependent protein kinase and protein kinase C exist in liver nuclei (78, 79).

Regucalcin regulates a process of signal transduction from the cytoplasm to the nucleus in liver cells. This process may be involved in various protein kinases and protein phosphatases. The activity of these enzymes is enhanced in the cytoplasm and nucleus of regenerating rat liver (36, 37, 45). Endogenous regucalcin has been demonstrated to have a suppressive effect on the enhancement of protein kinase and protein phosphatase activities in proliferative liver cells using anti-regucalcim monoclonal antibody (36, 37, 45). Regucalcin may inhibit tyrosine kinase, protein kinase C, and Ca^{2+}/calmodulin-dependent protein kinase in the cytoplasm and nucleus of regenerating rat liver (36, 37). Moreover, regucalcin has an inhibitory effect on protein tyrosine phosphatase and protein serine/threonine phosphatase activity in the cytoplasm and nucleus of regenerating liver (45).

Liver nuclear DNA and RNA synthesis is stimulated in regenerating liver. The nuclear DNA synthesis is markedly increased by partial hepatectomy. Regucalcin has been shown to inhibit DNA synthesis in the nuclei of normal and regenerating rat livers (52). Moreover, regucalcin can inhibit RNA synthesis in the nuclei of normal and regenerating rat livers (53). Ca^{2+} increases RNA synthesis in the nuclei of normal rat liver (53). Such an effect is potentiated in the nuclei of regenerating liver (53), suggesting that Ca^{2+} is required for nuclear RNA synthesis in proliferative liver cells. The stimulatory effect of Ca^{2+} is inhibited by α-amanitin, an inhibitor of RNA polymerase II. Regucalcin inhibits RNA synthesis in the nuclei of normal and regenerating rat livers. Interestingly, the presence of anti-regucalcin antibody has been shown to stimulate RNA synthesis, indicating that endogenous regucalcin is involved in nuclear RNA synthesis. Regucalcin may play an inhibitory role in the regulation of liver nuclear RNA synthesis. The role of regucalcin as a transcriptional factor in liver nuclei, however, remains to be elucidated. In addition, it has been shown that regucalcin has an inhibitory effect on protein synthesis in the cytoplasm in normal liver (47) and regenerating rat liver (48), and it can directly inhibit aminoacyl-tRNA synthetase activity in the cytoplasm.

As shown in Fig. 1, regucalcin plays an important role in the regulation of liver nuclear functions. Regucalcin mRNA expression in liver cells is stimulated through the pathway of signaling mechanism concerning Ca^{2+}-dependent protein kinases in regenerating liver. Regucalcin is transported to the nucleus of liver cells, and it reveals an inhibitory effect on Ca^{2+}-dependent protein kinases and protein phosphatases in the nuclei. Regucalcin, which is localized in liver nuclei, has an inhibitory effect on nuclear DNA and RNA synthesis in the proliferative cells of regenerating liver. Presumably, regucalcin plays a suppressive role in the overexpression of cell proliferation. Regucalcin is proposed to play an important role as a suppressor protein in the differentiation and proliferation of liver cells.

ROLE OF REGUCALCIN IN HEPATOMA CELLS

The expression of a variety of proteins is changed in tumour cells. In hepatoma, the reciprocal change of the level of albumin and α-fetoprotein is well known (80). The expression of some oncogenes is increased in hepatomas (81). Regucalcin mRNA expression is decreased in the tumorous tissues of liver of rats fed with 3′-methyl-4-dimetylaminoazobenzene, as compared with that of non-tumorous tissues in the liver (82). Also, regucalcin mRNA expression and its protein content are decreased in the cloned human hepatoma cells (Hep G2) (83) and rat hepatoma cells (H4-II-E) (71, 84). Interestingly, the existence of transcript heterogeneity of the human gene for regucalcin has been found in the cloned human hepatoma cell line (Hep G2) (57). These findings suggest that regucalcin is involved in the generation of hepatoma. The increase in protein kinase and protein tyrosine phosphatase activities is corresponded to the proliferation of cloned rat hepatoma cells (H4-II-E) (85), and endogenous regucalcin has a suppressive effect on the elevation of these enzymes activity in the cells (86), although regucalcin is expressed only slightly in the hepatoma cells. Presumably, the decrease in regucalcin leads to a acceleration of signal transduction from the cytoplasm to nucleus, and such a decrease weakens the suppressive effect of regucalcin on the overexpression of liver cell proliferation. Prolonged reduction of regucalcin expression may induce a generation of hepatoma. In fact, the decrease in regucalcin mRNA expression has been demonstrated in the liver of rats administered with phenobarbital (86), carbon tetrachloride (87), galactosamine (88), ethanol (89), and streptozotocin-diabetic state (89), which cause a disorder of liver metabolism.

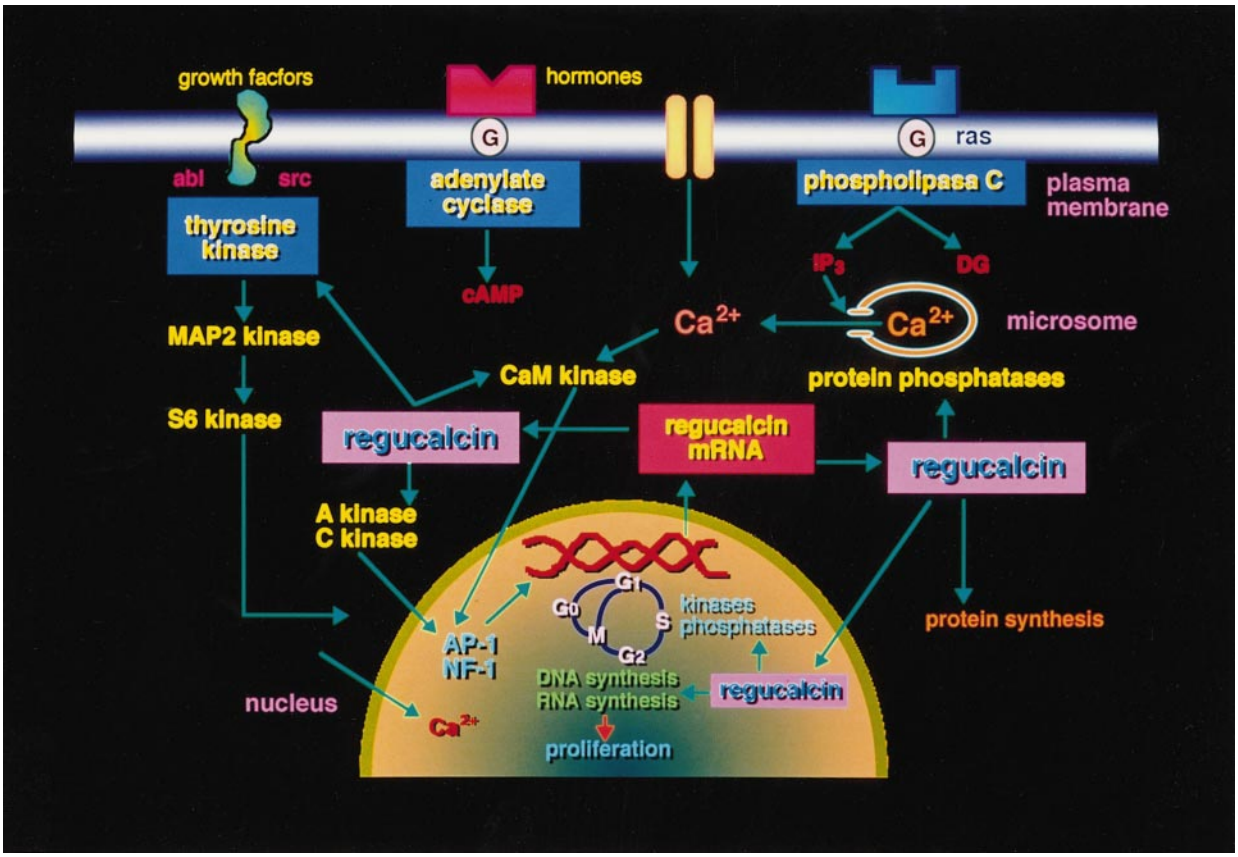

FIG. 1. Role of regucalcin in the regulation of nuclear functions in regenerating rat liver with a proliferative cells. Regucalcin mRNA expression is stimulated through the pathway of signaling mechanism concerning Ca^{2+}/calmodulin-dependent protein kinase (CaM kinase), protein kinase C, and tyrosine kinase increased by partial hepatectomy. Regucalcin inhibits the activities of nuclear protein kinases and protein phosphatases, and it inhibits nuclear DNA and RNA synthesis. Moreover, regucalcin inhibits protein synthesis in the cytoplasm of regenerating liver. By such a mechanism, regucalcin may suppress the overexpression of proliferation of liver cells.

PROSPECTS

Regucalcin, which was discovered as a Ca^{2+}-binding protein in 1978, has been shown to play a multifunctional role in liver and kidney cells. As introduced in this review, regucalcin has been found to regulate the nuclear functions in regenerating liver with proliferative cells. Regucalcin has also been demonstrated to be expressed in bran neuronal cells (90, 91), suggesting its physiologic role in brain function. More important role of regucalcin may be found out in other tissues and cells. In addition, growing evidence is that regucalcin may be implicated to pathophysiologic state in liver injury (86–89), hypertensive state (92) and Alzheimer's disease (90), which regucalcin expression is reduced. Further studies are expected to determine a clinical aspect of regucalcin for disease.

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