Regucalcin, an inhibitor of cell signaling and transcription activity, is involved in suppression of human carcinogenesis

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Abstract

Regucalcin was discovered in 1978 as a calcium-binding protein. After that, regucalcin was demonstrated to play a multifunctional role as a suppressor protein in signal transduction in various types of cell and tissues. The regucalcin gene (rgn) is localized on the X chromosome. Regucalcin was found to suppress nuclear deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis in liver cells. Overexpression of endogenous regucalcin possessed suppressive effects on proliferation in the modeled rat hepatoma cells by inhibiting G1 and G2/M cell cycle arrests. Suppressed regucalcin gene expression was found to be associated with progression of hepatocarcinogenesis by proteosome analysis. Moreover, regucalcin mRNA expression was found to suppress in various human normal and tumor tissues including hepatocellular carcinoma, kidney transitional cell carcinoma, brain malignant meningioma, and lung non-small cell carcinoma of human subjects. Suppressed regucalcin gene expression may be a key in development of carcinogenesis. Development of the regucalcin gene deliver system will be expected as a novel gene therapy in clinical aspects for cancer treatment.

Keywords: regucalcin, cell signaling, nuclear regulation, cell proliferation, carcinogenesis

Introduction

Regucalcin was discovered in 1978 as a novel calcium-binding protein that suppresses calcium signaling in various types of cells and tissues [1-6]. The regucalcin gene (rgn) is localized on X chromosome [7, 8], and organization of the regucalcin gene consists of seven exons and six introns [9]. Regucalcin are identified in over 15 species consisting of regucalcin family in vertebrate and invertebrate species [5, 6, 10]. Various transcription factors have been shown to enhance transcription activity of the regucalcin gene expression that is mediated through Ca²⁺ and other signal systems [10]. Regucalcin plays a multifunctional role in cell regulation; maintaining of intracellular Ca²⁺ homeostasis, suppressions of signal transduction, protein synthesis, cell proliferation and apoptosis [2-4]. Regucalcin has been proposed to play an important role in maintaining cell homeostasis as a suppressor protein in cell signaling in various types of cells and tissues [2-4].

Cell proliferation is mediated through various intracellular signaling transductions that are stimulated by many hormone and cytokines. Enhanced cell proliferation may lead to carcinogenesis. However, mechanism of carcinogenesis is complexity and its therapy is not established. Cancer is a pathological condition, where assemblage of cells displays uncontrolled growth, invasion and metastasis. Regucalcin is a novel suppressor protein in cell signaling [2-4]. Regucalcin is demonstrated to play a multifunctional role in cell regulation in various types of cell and tissues [2-4]. Regucalcin is predominantly expressed in liver and kidney tissues, although it is expressed in other many tissues [10]. Interestingly, overexpression of the regucalcin gene was found to suppress liver cell proliferation and carcinogenesis in animal models [11-13]. Moreover, there is growing evidence that the regucalcin gene expression is uniquely suppressed in various human carcinoma tissues using analysis with multiple gene expression profiles and proteomics [11-14]. Suppressed regucalcin gene expression may lead to development of carcinogenesis. This review focuses a potential role as a suppressor protein of regucalcin in the development of human carcinogenesis.

Role of regucalcin in nuclear regulation and cell proliferation

Regucalcin is present in the cytoplasm in cells, and it is translocated into the nucleus. Nuclear translocation of regucalcin...
is not regulated through adenosine 5'-triphosphate and guanosine 5'-triphosphate, which are required for nuclear import of proteins [15]. Nuclear translocation of regucalcin was not related to nuclear localization signal that is responsible for selection for intranuclear active transport [16]. Regucalcin may be passively transported to the nucleus through nuclear pore in cells, since the molecular weight of regucalcin is about 33 kDa [5]. Regucalcin has also been shown to localize in the nuclei of the cloned normal rat kidney proximal tubular epithelial NRK52E cells with immunocytochemical analysis [17]. Nuclear localization of regucalcin is enhanced through hormonal Ca²⁺-signaling dependent process that is involved protein kinase C [17]. Regucalcin has been shown to bind protein and DNA in the nucleus [18]. Regucalcin has been shown to regulate various enzyme activities in the nucleus. Endonuclease is responsible for DNA fragmentation occurring during programmed cell death (apoptosis) and certain forms of chemically induced cell killing [19]. Regucalcin has been found to have suppressive effects on Ca²⁺-activated DNA fragmentation due to inhibiting endonuclease activity in isolated rat liver nuclei [20]. Smal GTPase Ran (ras-related nuclear protein) is required for protein export from the nucleus and protein import into the nucleus [21]. Regucalcin inhibits GTPase activity in rat liver nucleus [15]. Process of signal transduction from the cytoplasm to nucleus in liver cells is mediated through various protein kinases and protein phosphatases. Regucalcin is found to suppress the activities of tyrosine kinase, protein kinase C and Ca²⁺/calmodulin-dependent protein kinase, which are enhanced in the cytoplasm and nucleus obtained from regenerating rat liver with proliferating cells in vivo [22]. The activity of nuclear Ca²⁺-dependent protein kinases has been shown to increase in the presence of anti-regucalcin monoclonal antibody in the enzyme reaction mixture, and such increases are completely depressed with addition of regucalcin [22]. In addition, nuclear endogenous regucalcin has been shown to play a suppressive role in the regulation of protein tyrosine phosphatases using anti-regucalcin monoclonal antibody in the reaction mixture [23]. Thus, regucalcin has been shown to play a pivotal role in the regulation of the activity of various enzymes in the nucleus.

Regucalcin has also been shown to have suppressive effects on DNA and RNA synthesis activity in the nuclei of normal rat liver and regenerating rat liver in vivo [24-27]. Regucalcin may have suppressive effects on the enhancement of nuclear DNA and RNA synthesis in proliferating liver cells in vivo. Also, regucalcin has a suppressive effect on DNA synthesis activity in the nuclei isolated from rat renal cortex in vitro [28]. The presence of anti-regucalcin monoclonal antibody in the reaction mixture causes an increase in nuclear DNA synthesis activity [24, 25]. This increase was completely depressed in the presence of regucalcin. Thus, endogenous regucalcin is found to have a suppressive effect on DNA synthesis in the nuclei of rat liver and renal cortex [24, 25]. The effect of regucalcin in decreasing nuclear RNA synthesis activity in normal rat liver is not seen in the presence of α-amanitin, an inhibitor of RNA polymerase II and III [26, 27], suggesting that its suppressive effect is partly resulted from the inhibitory action on RNA polymerase II and III. Regucalcin may have direct inhibitory effects on nuclear DNA and RNA polymerase activity. Moreover, regucalcin has been shown to regulate nuclear function in proliferating cells using cloned hepatoma H4-II-E cells which were cultured in the presence of fetal bovine serum (FBS). Culture with FBS produced an increase in cell number and a corresponding elevation of various kinase activities, which are related to Ca²⁺/calmodulin-dependent protein kinase, protein kinase C, protein tyrosine kinase and protein phosphatase activity in H4-II-E cells [29-31]. These enzymes may contribute to the enhancement of hepatoma cell proliferation after serum stimulation. The presence of anti-regucalcin monoclonal antibody in the enzyme reaction mixture using H4-II-E cells cultured with FBS stimulation was found to increase the activities of protein kinase and protein phosphatase. Such an effect was depressed after addition of exogenous regucalcin in the enzyme reaction mixture. Regucalcin may play an important role as a suppressor in the enhancement of cell proliferation due to inhibiting the activities of various protein kinases and protein phosphatases in the cytoplasm and nucleus [29-31]. In addition, nuclear DNA synthesis activity has been shown to increase at 6 hours after culture with FBS, which is preceded an elevation of the number of H4-II-E cells cultured with FBS [32, 33]. The presence of regucalcin in the reaction mixture suppressed nuclear DNA synthesis activity in the cells. This effect may be partly mediated through pathway of various protein kinases in H4-II-E cells. Endogenous regucalcin has been shown to suppress DNA synthesis activity through mechanism by which inhibits protein kinases in the nuclei of proliferating H4-II-E cells using anti-regucalcin monoclonal antibody [32]. To determine the role of endogenous regucalcin in the regulation of nuclear DNA synthesis, regucalcin/pCXN2-transfected cells, which H4-II-E cells overexpress regucalcin stably, have been generated [33]. The increase in cell number and DNA synthesis activity in transfectants was found to suppress as compared with those of wild- and mock-type, indicating that overexpression of endogenous regucalcin has suppressive effects on cell proliferation [33]. The presence of anti-regucalcin monoclonal antibody in the reaction mixture caused increases in DNA synthesis activity in the nuclei obtained from wild-type H4-II-E cells, mock-type cells, and transfectants with overexpression of regucalcin [33]. However, such an increase was remarkable in transfectants [33]. This finding supports the view that the augmentation of endogenous regucalcin has great suppressive effects on nuclear DNA synthesis activity in proliferating hepatoma cells. Regucalcin may play a suppressive role for the over-proliferation of liver cells.

Whether regucalcin suppresses cell cycle-realted genes in proliferating cells has been examined. Overexpression of regucalcin is found to induce G1 and G2/M phase cell cycle arrest in transfectants (H4-II-E cells) [34]. p21 mRNA expression was found to enhance in transfectants, although cdc2a and chk2 (checkpoint-kinase 2) mRNA levels were not changed [34]. p21 is an inhibitor of cyclin-dependent kinases (cdk). Regucalcin may
enhance p21 expression and inhibits G1 progression in H4-II-E cells. Overexpression of endogenous regucalcin has also been shown to suppress proliferation of cloned normal rat kidney proximal tubular epithelial NRK52E cells [35]. Endogenous regucalcin is found to induce G1 and G2/M phase cell cycle arrest in NRK52E cells [35]. Expression of c-jun and chk2 (checkpoint-kinase 2) mRNAs was also suppressed in the transfectants of NRK52E cells [35]. The expression of c-myc, c-fos, cdc2, and p21mRNAs was not changed in transfectants [35]. Decrease in c-jun and chk2 mRNA expressions may partly contribute to suppression of cell proliferation induced in regucalcin-overexpressing NRK52E cells. c-myc, c-fos, c-jun, and Ha-ras are known as tumor stimulator genes [36]. p53 and Rb are tumor suppressor genes, and c-src is oncogene [37]. Expression of c-myc, Ha-ras or c-src mRNAs was found to suppress in regucalcin-overexpressing transfecants [38]. Expression of p53 and Rb mRNAs was markedly enhanced in transfecants [38]. Suppressed expression of c-myc, Ha-ras and c-src mRNAs and enhanced expression of p53 and Rb mRNAs in transfecants may be partly involved in retardation of proliferation of hepatoma H4-II-E cells. Also, expression of p53 mRNA was enhanced in regucalcin-overexpressing transfecants of NRK52E cells, while expression of c-myc, c-fos, cdc2, and p21mRNAs was not changed in transfecants [35]. Decrease in c-jun and chk2 mRNA expressions may partly contribute to suppression of cell proliferation induced in NRK52E cells overexpressing regucalcin. p53 mRNA expression, which was enhanced in transfecants, may play a role in retardation of proliferation of NRK52E cells.

Thus, regucalcin has been shown to have suppressive effects on cell proliferation due to regulating many gene expressions that are related to cell proliferation in hepatoma H4-II-E cells and normal kidney NRK52E cells [39]. Regucalcin can bind DNA and modulates nuclear transcriptional activity [18]. Regucalcin may bind to the promoter region of various genes which suppress stimulator gene expression or stimulate suppressor gene expression in cell proliferation [39]. As the result, overexpression of endogenous regucalcin suppresses cell proliferation [39]. Regucalcin may play an important role as a suppressor protein in cell proliferation.

Role of regucalcin in rescue of apoptotic cell death

Regucalcin has been shown to regulate gene expression of proteins which are related to apoptosis [40]. Overexpression of regucalcin has a suppressive effect on apoptotic cell death induced by tumor necrosis factor (TNF)-α, lypopolyssacharide, Bay K 8644, or thapsigargin in NRK52E cells, suggesting that its suppressive effects may be mediated through many intracellular signaling pathways in NRK52E cells [41]. Bcl-2 is a suppressor protein in apoptotic cell death [42]. Apaf-1 participates in activation of caspase-3 [43]. Akt-1 is involved in survival signaling pathway for cell death [43]. Overexpression of regucalcin caused a remarkable elevation of Bcl-2 mRNA expression in NRK52E cells, and it slightly stimulated Akt-1 mRNA expression in the cells [44]. The expression of Apaf-1 or caspase-3 mRNAs was not changed in transfecants [41]. The enhancement of Bcl-2 mRNA expression may contribute to suppression of apoptotic cell death in NRK52E cells. Regucalcin may play a role in the regulation of Bcl-2 gene expression in NRK52E cells. Regucalcin regulates the expression of Bcl-2, caspase-3, and Akt-1 mRNAs in NRK52E cells. Thus, regucalcin rescues apoptosis that is mediated through gene expression of protein molecule which is related to cell apoptosis. Interestingly, overexpression of regucalcin has been found to have suppressive effects on the remarkable increase in α-smooth muscle actin level in NRK52E cells cultured with TNF-α or transforming growth factor (TGF)-β1 [44]. This finding suggests that regucalcin regulates signal pathway that is mediated through TNF-α or TGF-β1 to stimulate α-smooth muscle actin expression. TGF-β1 is a key mediator that regulates transdifferentiation of NRK52E cells into myofibroblasts due to expressing α-smooth muscle actin which is contributed to renal fibrosis associated with overexpression of TGF-β1 within diseased kidney [45]. Regucalcin may regulate transdifferentiation to renal fibrosis in NRK52E cells with TGF-β1 or TNF-α. In addition, overexpression of regucalcin caused a remarkable increase in Smad 2 mRNA expression, which is involved in signal transduction of TGF-β1 [45], and NF-κB mRNA expression, which is related to signal of TNF-α [46], in NRK52E cells. Regucalcin may have suppressive effects on signal pathway by which TNF-α or TGF-β1 stimulates gene expression of NF-κB or Smad 2 in NRK52E cells.

As described above, regucalcin has been shown to translocate into the nucleus of various cell types. Regucalcin binds nuclear proteins and DNA, and it suppresses DNA and RNA synthesis and the phosphorylation and dephosphorylation of various proteins which are related to transcription. In addition, regucalcin may directly bind on the promoter region of gene and regulates the expression of various genes as a transcription factor. Regucalcin may be a key molecule in cell nuclear regulation. Cellular and molecular mechanisms by which regucalcin suppresses cell proliferation is summarized in (Figure 1).

Role of regucalcin in the suppression of carcinogenesis

Hepatocellular carcinoma (HCC), the most common primary liver cancer, is one of the most prevalent malignant diseases worldwide, and the third most common causes of cancer-related death [47-49]. Globally, there are approximately 750,000 new cases of HCC reported per year. The incidence of HCC is increasingly in the United States and other developed countries. Moreover, features of HCC are an aggressive cancer with a dismal outcome largely due to metastasis and postsurgical recurrence. In most cases, HCC originates on a background of cirrhosis, a chronic and diffuse hepatic disease that results from continuous liver injury and regeneration [49]. Cirrhosis is present in approximately 80%-90% of HCC patients and constitutes the largest single risk factor. In cirrhotic liver, changes in fat metabolism associated with the activation of adipocyte-like pathways are thought to be involved in neoplastic transformation [49]. Increased hepatocyte turnover, inflammation and oxidative DNA damage is implicated in the pathogenesis of the liver disease including obesity, Type 2 diabetes,
insulin resistant and nonalcoholic fatty liver disease. The prevalent risk factors for HCC are also the cause of liver cirrhosis that includes viral infections (hepatitis B and C) and alcohol consumption; further risk factors include tobacco smoking, exposure to aflatoxin B1 and vinyl chloride, diabetes, and genetic disorders, such as hemochromatosis and alpha-1 antitrypsin deficiency [50-54].

Hepatocarcinogenesis is a multistep process initiated by external stimuli that lead to genetic changes in hepatocytes or stem cells, resulting in proliferation, apoptosis, dysplasia and neoplasia. The majority of HCC cases are also related to chronic viral infections. Hepatitis B virus (HBV) DNA integrates into the host genome, inducing chromosome instability and insertional mutations that may activate various oncogenes, such as cyclin A [55-58]. Viral proteins, in particular X protein (HBx), act as transactivators to upregulate several oncogenes (such as c-myc and c-jun) and transcriptional factors (such as nuclear factor-kB) [59-61]. Additionally, HBx activates promoters of genes encoding interleukin-8 (IL-8), tumor necrosis factor (TNF), transforming growth factor (TGF)-β and epidermal growth factor receptor (EGFR) [62]. HBx can also stimulate several signal transduction pathways, including the JAK/STAT, RAS/RAF/MAPK, and Wnt/β-catenin pathways [62, 63]. The contributions of hepatitis C virus (HCV) to hepatocarcinogenesis are mediated through viral proteins, including core, NS3 and NS5A proteins. HCV core protein can promote apoptosis or cell proliferation through interaction with p53 or upregulation of Wnt-1 at the transcriptional level [64-66].

The prognosis of advanced HCC remains poor in spite of the development of novel therapeutic strategies [67]. Traditional therapies are not effective for HCC and are too toxic for patients with cirrhosis. Transarterial chemoembolization and radioembolization are the main treatments for intermediate-stage HCC at the present time. Improved knowledge of the oncogenic processes and signaling pathways, which regulate tumor cell proliferation, differentiation, angiogenesis, invasion and metastasis, has led to the identification of several potential therapeutic targets that have driven the development of molecularly targeted therapies [67]. An ideal cancer target meets the following criteria: the target is relatively specific for cancer cells (not expressed or expressed at very low levels in normal cells but overexpressed in cancer cells).
Meanwhile, overexpression of the target is associated with malignant biological phenotypes and/or poor prognosis; the target plays an essential role in cancer initiation and progression, and inhibition of expression or activity of the target induces growth suppression and/or apoptosis in cancer cells. The target is “drugable” as an enzyme (e.g., a kinase) or cell surface molecule (e.g., a membrane-bound receptor) that can be easily screened for small-molecule inhibitors or targeted by a specific antibody [67, 68]. The only systemic therapy available for advanced HCC is based on the multikinase inhibitor sorafenib [68], which is the most effective therapeutic tool for advanced unresectable HCC. The survival of patients with advanced HCC treated with sorafenib depends on the absence of liver dysfunction and on the status of the patient [69]. In the past few years, the use of sorafenib in combination with transarterial chemoembolization has improved survival rates in patients with advanced HCC. Recently, new perspectives in cancer treatment have appeared with the advent of microRNAs, a novel class of noncoding small RNAs [70].

Regucalcin may play a pivotal role in the suppression of hepatocarcinogenesis [35, 71]. Regucalcin plays a role as a suppressor protein in various cell signal transductions [3, 4]. Overexpression of regucalcin was found to play a role as a suppressor protein in cell proliferation that is mediated through various signaling stimulations in the cloned normal rat kidney proximal tubular epithelial NRK52E cells and the cloned rat hepatoma H4-II-E cells [11]. Regucalcin caused G1 and G2/M phase cell cycle arrest in these cells [11]. The anti-cell proliferation effect of regucalcin was not dependent on apoptosis; regucalcin suppresses apoptosis induced through multifaceted signaling pathways [40]. Molecular mechanisms by which regucalcin suppresses the promotion of cell proliferation was elucidated. Regucalcin directly inhibited the activities of various Ca²⁺/calmodulin-dependent enzymes, protein kinases and protein phosphatases in the cytoplasm and nuclei [3, 4]. Nuclear regucalcin was found to inhibit nuclear DNA and RNA synthesis and suppress the gene expression of c-my, Ha-ras and c-src, a tumor-stimulator gene, and stimulate the gene expression of p53 and Rb, a tumor-suppressor gene [39]. Moreover, regucalcin was demonstrated to inhibit protein synthesis due to inhibiting aminoacyl-tRNA synthetase and stimulate protein degradation due to activating cysteiny1 protease [3, 4]. Thus, suppressive effects of regucalcin on cell proliferation are mediated through molecules with multi targets in liver cells.

The gene expression of regucalcin was found to suppress in hepatocarcinogenesis. Liver regucalcin gene expression was suppressed at earlier periods of carcinogenesis in rats treated with diethylnitrosamine and then 2-acetylaminofluorene combined with partial heptectomy, which induces an increase in proliferating cells [12]. The suppression of regucalcin protein expression was identified by proteomic analysis that was differentially expressed in the livers of rats fed 5% ethanol for 1 and 3 months [13]. Liver regucalcin mRNA expression was suppressed by disorder of liver metabolism induced by administration of carbon tetrachloride [72], galactosamine [73] and phenobarbital [74] in rats. In addition, liver regucalcin level was reduced with the conditions of diabetes and ethanol ingestion [75] that lead to cirrhosis and HCC. The suppression of regucalcin gene expression may lead to the development of HCC. Noticeably, the regucalcin gene and its protein levels was found to specifically suppress in human HCC using analysis with multiple gene expression profiles and proteomics [76-80]. Suppressed regucalcin gene expression may lead to development of human hepatocarcinogenesis.

Prospects

Recently, we have demonstrated that regucalcin mRNA expression is suppressed in various human normal and tumor tissues including hepatocellular carcinoma, kidney transitional cell carcinoma, brain malignant meningioma, and lung non-small cell carcinoma evaluated with clinical diagnosis of human subjects [14]. Regucalcin may be a key molecule as a suppressor in cell proliferation and carcinogenesis in various types of cells and tissues. Overexpression of the regucalcin gene in cancer cells may possess preventive and therapeutic effects on the development of carcinogenesis. Development of the regucalcin gene delivery system will be expected as a novel gene therapy in clinical aspects for cancer treatment.

Author disclosures

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