

A potential role as a suppressor protein of Regucalcin in the development 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 proteasome 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 delivery 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]. Small 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 antiregucalcin 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 antiregucalcin 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]. 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]. 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 [30].

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 smallmolecule 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 nonresectable 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 multisignaling pathway [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-myc, Ha-ras and c-src, a tumor-stimulator gene, and stimulate the gene expression of p53 and Rb, a tumor-suppressor gene [31]. Moreover, regucalcin was demonstrated to inhibit protein synthesis due to inhibiting aminoacyl-tRNA synthetase and stimulate protein degradation due to activating cysteinyl 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 hepatectomy, which induces an increase in proliferating cells [12]. The suppression of regucalcin protein expression was identified in 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 in rats. In addition, liver regucalcin level was reduced with the conditions of diabetes and ethanol ingestion 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 nonsmall 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 deliver system will be expected as a novel gene therapy in clinical aspects for cancer treatment.

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