

The Regucalcin Gene is a Key in the Therapy of Hepatocellular Carcinoma

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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 [1-3]. 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 result from continuous liver injury and regeneration [3]. 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 [3]. 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, and include 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 [4-8].

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 [9-12]. 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- κ B) [13-15]. 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) [16]. HBx can also stimulate several signal transduction pathways, including the JAK/STAT, RAS/RAF/MAPK, and Wnt/ β -catenin pathways [16,17]. 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 [18-20].

The prognosis of advanced HCC remains poor in spite of the development of novel therapeutic strategies [21]. 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 that regulate tumor cell proliferation, differentiation, angiogenesis, invasion and metastasis has led to the identification of several potential therapeutic targets, which have driven the development of molecularly targeted therapies [21]. 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) [21]. 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 [21,22]. The only systemic therapy available for advanced HCC is based on the multikinase inhibitor sorafenib [22], which is the most effective therapeutic tool for advanced nonresectable HCC, in which it can slightly improve patient survival. The survival of patients with advanced HCC treated with sorafenib depends on the absence of liver dysfunction and on the status of the patient [23]. 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 [24].

Regucalcin, which the author discovered in 1978 [25-28], may play a pivotal role in the suppression of hepatocarcinogenesis [29-31]. Regucalcin plays a role as a suppressor protein in various cell signal transductions [26-28]. The regucalcin gene is located on the X chromosome in consisting of seven exons and six introns [32]. Regucalcin (RGN) and its gene (*rgn*) are identified in over 15 species consisting of regucalcin family and the gene species are highly conserved in vertebrate species [32]. The regucalcin gene expression is regulated through various transcription factors (including AP-1, NF1-A1, RGPR-p117, β -catenin, SP1 and others), which are identified as the enhancer and suppressor, and this expression is regulated with hormonal stimulation and physiological state [32]. Regucalcin plays a pivotal role as a suppressor protein in various signal transductions to maintain cell homeostasis for stimuli, and it plays a multifunctional role in cell regulation through maintaining of intracellular Ca^{2+} homeostasis and suppressing of signal transduction in various cell types [26-28]. Interestingly, the cytoplasmic regucalcin is translocated into the nucleus and inhibits nuclear Ca^{2+} -dependent and -independent protein kinases and protein phosphatases, Ca^{2+} -activated endonuclease, and DNA and RNA synthesis [33].

Regucalcin has been demonstrated to play a role as a suppressor protein in cell proliferation, which is mediated through various signaling stimulations, in the cloned normal rat kidney proximal

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tubular epithelial NRK52E cells and the cloned rat hepatoma H4-II-E cells [31,34,35]. Regucalcin causes G1 and G2/M phase cell cycle arrest in these cells [31,36]. The anti-cell proliferation effect of regucalcin is not dependent on apoptosis; regucalcin suppresses apoptosis induced through multisignaling pathway [37]. Molecular mechanisms by which regucalcin suppresses the promotion of cell proliferation have been elucidated. Regucalcin, which is expressed through signaling factors that stimulate cell proliferation, is translocated into the nucleus with mechanism by which is mediated through protein kinase C-dependent signaling [31,34]. Regucalcin directly inhibits the activities of various Ca^{2+} /calmodulin-dependent enzymes, protein kinases and protein phosphatases in the cytoplasm and nuclei [27,28,31]. Regucalcin inhibits nuclear DNA and RNA synthesis, and it has suppressive effects on the expression of *c-myc*, *Ha-ras*, and *c-src* mRNA, which are tumor-stimulator genes and also stimulates the expression of *p53* and *Rb* mRNAs that are tumor-suppressor genes [31,38]. Moreover, regucalcin inhibits protein synthesis and stimulates protein degradation due to inhibiting aminoacyl-tRNA synthetase and activating cysteinyl protease [27,28]. Thus, suppressive effects of regucalcin on cell proliferation are mediated through the suppression of many cell signaling processes including the suppression of oncogene expression and stimulation of tumor suppressor gene expression in liver cells [31,33,38].

Noticeably, the regucalcin gene and its protein levels have been found to specifically suppress in human hepatocellular carcinoma (HCC) using analysis with multiple gene expression profiles and proteomics [39-43]. The suppression of regucalcin gene expression has been shown to occur 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 [29]. The suppression of regucalcin protein expression has also been identified in proteomic analysis that was differentially expressed in the livers of rats fed 5% ethanol for 1 and 3 months [30]. In addition, regucalcin mRNA expression is suppressed by disorder of liver metabolism (including carbon tetrachloride [44], galactosamine [45] and phenobarbital [46] administration, the conditions of diabetes and ethanol ingestion [47]), which may lead to cirrhosis and HCC. The suppression of regucalcin gene expression may lead to the development of HCC.

Regucalcin, a suppressor protein in cell signaling system, may play a role as a key molecule in the depression of cell proliferation and carcinogenesis of various tissues and cell types. Overexpression of regucalcin in cancer cells may play preventive and therapeutic roles in carcinogenesis. The development of a novel gene therapy with the regucalcin gene deliver system will be expected in clinical aspects.

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