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Novel Protein RGPR-p117: New Aspects in Cell Regulation

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Abstract

RGPR-p117 was initially discovered as novel protein which binds to the nuclear factor I (NF1)-like motif TTGGC(N)$_6$CC in the regucalcin gene promoter region (RGPR). RGPR-p117 is localized to the nucleus with stimulation of protein kinase C-related signaling process. Overexpression of RGPR-p117 has been shown to enhance regucalcin mRNA expression in the cloned normal rat kidney proximal tubular epithelial NRK52E cells *in vitro*. This process is mediated through phosphorylated RGPR-p117. Overexpression of RGPR-p117 was found to suppress apoptotic cell death induced after stimulation with various signaling factors in NRK52E cells, while it did not have an effect on cell proliferation. Moreover, RGPR-p117 was found to localize in the plasma membranes, mitochondria and microsomes, suggesting an involvement in the regulation of function of these organelles. After that, RGPR-p117 was renamed as Sec16B that is involved in the endoplasmic reticulum export. However, this is not suitable name with many findings of the role of RGPR-p117 in cell regulation. RGPR-p117 may play an essential role as transcription factor, and the elucidation of other roles in cell regulation will be expected.

Keywords: RGPR-p117; Regucalcin; Transcription factor; Sec16B; Apoptosis

Introduction

RGPR-p117 is a novel transcription factor, which was initially discovered in 2001 as the regucalcin gene promoter region-related protein (RGPR) [1]. Regucalcin has been shown to play a multifunctional role in cell regulation; regulation of intracellular Ca^{2+} homeostasis, suppressions of cell signaling process, protein synthesis, nuclear deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis, cell proliferation and apoptotic cell death in many cell types [2-5]. The regucalcin gene localizes on X chromosome [6,7]. The promoter region of the regucalcin gene contains a nuclear factor I (NF1)-like motif TTGGC(N)$_6$CC which is the nuclear factor binding site [8,9]. RGPR-p117 was identified as a transcription factor that binds to the TTGGC motif of the regucalcin gene using a yeast one-hybrid system [1]. This short communication will discuss a role of RGPR-p117 in cell regulation.

Role of RGPR-P117 as Transcription Factor

We produced a full-length cDNA of this novel gene with a RACE-PCR method and found a novel regucalcin gene promoter region-related protein [1]. The length of this cDNA (4378 bp) corresponded to approximately 4.5 kb band as observed with Northern hybridization [1]. This protein was termed as a regucalcin gene promoter region-related protein [1]. The human RGPR-pl 17 gene is located in chromosome Iq 25.2 and consists of 26 exons spanning approximately 4. 1 kbp [1]. The entire human RGPR-p117 cDNA consists of 3,989 bp, which contains an open reading frame (ORF) of 3,180 bp encoding a protein of 1,060 amino acid residues [1]. RGPR-p117 is identified in human, rat, mouse, bovine, rabbit and chicken [1,10]. The RGPR-p117 gene is found in dog, cow, pig, frog (*Xenopus*), fish (*Zebrafish*), *C. elegance* and yeast thus far [8]. Phylogenetic analysis of six vertebrates shows that RGPR-p117 appears to form a single cluster, indicating a common evolutionary relationship of the RGPR-p117 family [10]. RGPR-p 117 in rat, mouse and human is consisted of 1058, 1051, and 1060 amino acid residues with calculated molecular mass of 117, 115, and 117 kDa and estimated pI of 5.69, 5.70, and 5.71, respectively [1-10]. The homologies of amino acids among rat, mouse and human RGPR-p 117 were at least 70%. Mammalian RGPR-p117 conserves a leucine zipper motif [1], which is present in many gene regulatory proteins, such as CCATT-box and enhancer binding protein (C/EPP) [11-13], nuclear oncogenes fos and jun [14], cyclic AMP response clement (CRE) binding proteins (CREB; CRE-BPI, ATFs) [15], C-myc, L-myc and N-myc oncogenes [16] and octamer-binding transcription factor 2 (Oct-2/0TF-2) [17]. RGPR-p117 may play a pivotal role as a transcription factor in gene expression.

RGPR-p117 mRNA is expressed in the liver, kidney, heart, spleen, and brain of rats [1]. The sexual difference of this expression is not found [18]. Liver RGPR-p117 mRNA expression is not changed with increasing age and was not altered by fasting or refeeding [18]. Regucalcin mRNA expression is stimulated through various signaling mechanisms, which were related to Ca^{2+}, cyclic adenosine monophosphate, protein kinase C, insulin, estrogen and other [19]. Computer analysis of subcellular localization of RGPR-p117 from six vertebrates showed a higher probability of nuclear localization especially in rats and mice (78.3%) [1,11]. The nuclear localization of RGPR-p117 has been demonstrated using the cloned normal rat kidney proximal tubular epithelial NRK52E cells *in vitro* [20]. RGPR-p117 has been shown to localize from the cytoplasm to nucleus which is enhanced through Ca^{2+} signaling-dependent protein kinase C in NRK52E cells [20]. RGPR-p117 in the nucleus may be phosphorylated by various protein kinases including protein kinase C [20]. Phosphorylated RGPR-p117 in the nucleus binds to the TTGGC motif in the promoter region of the regucalcin gene [20]. RGPR-p117 has been shown to enhance the expression of regucalcin mRNA in the nucleus of NRK52E cells [21]. The stimulatory effect of RGPR-p117 on regucalcin mRNA expression in NRK52E cells was not seen in mutant cells which are deleted the TTGGC motif [22]. RGPR-p117 has been demonstrated to play a role as a transcriptional factor in the enhancement of regucalcin

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gene expression in the cells. Thus, RGPR-p117 plays a pivotal role as a transcriptional factor. Moreover, RGPR-p117 may regulate other gene expressions as a transcription factor. RGPR-p117 binds to the TTGGC(N)6CC motif [1]. There are many genes that contain the TTGGC motif in the promoter region including regucalcin, albumin, glucokinase, a-fetoprotein, adenylate cyclase, phosphoenolpyruvate carboxykinase and others [23]. RGPR-p117 appears to regulate the expression of many genes.

Role of RGPR-p117 as Suppressor in Apoptosis

To elucidate the role of RGPR-p117 in cell regulation, we generated stable RGPR-p117/phCMV2-transfected NRK52E cells (transfectants) that overexpress endogenous RGPR-p117 [24]. Overexpression of RGPR-p117 did not cause a significant change in the proliferation of NRK52E cells, which were cultured in the presence of bovine serum including many hormones and cytokines [24]. However, overexpression of RGPR-p117 has been found to cause a significant decrease in protein and DNA contents in NRK52E cells [24], suggesting that RGPR-p117 has suppressive effects on protein and DNA synthesis or stimulatory effects on their degradation in NRK52E cells. Moreover, overexpression of RGPR-p117 has been shown to have suppressive effects on cell death induced after culture with tumor necrosis factor-α (TNF-α), lypopolysaccharide (LPS) or Bay K 8644 in NRK52E cells [25]. These factors-induced cell deaths were significantly suppressed in the presence of the caspase-3 inhibitor in NRK52E cells [25]. TNF-α- or LPS-induced DNA fragmentation in the cells was also suppressed in RGPR-p117-overexpressing NRK52E cells [25]. Thus, RGPR-p117 has been shown to have suppressive effects on apoptotic cell death [25]. This suggests that RGPR-p117 regulates various signaling processes. Moreover, overexpression of RGPR-p117 has been found to induce a decrease in mRNA levels of Fas-associating death domain protein (FADD), caspase-8, caspase-9, or caspase-3 which is involved in the stimulation of apoptotic cell death in NRK52E cells [25]. RGPR-p117 may have suppressive effects on apoptotic cell death due to decreasing the gene expression of various proteins which are related to stimulation of apoptosis. The TTGGC motif, which was found in the promoter region of the rat regucalcin gene, is present in the promoter region of the genes of caspase-3, caspase-8, or FADD as shown in the Databases [25]. The expression of these genes was found to suppress in RGPR-p117-overexpressing cells [25]. RGPR-p117 may bind to the TTGGC motif in the promoter region of these genes in NRK52E cells and may suppress the gene expressions of caspase-3, caspase-8, or FADD in the cells. In addition, the death of NRK52E cells has been shown to induce after culture with thapsigargin, which is an inhibitor of Ca^{2+} pump (Ca^{2+}-ATPase) in the endoplasmic reticulum (Ca^{2+} store) of various cell types [26,27]. Thapsigargin-induced cell death was not suppressed with overexpression of RGPR-p117 in the cells [25]. RGPR-p117 may not regulate signaling mechanism of cell death induced through thapsigargin. Thapsigargin-induced apoptotic cell death is related to an increase in intracellular Ca^{2+} concentration [26]. This increase activates nuclear Ca^{2+}-dependent endonuclease to mediate DNA cleavages into oligonucleosome fragments [27,28]. RGPR-p117 may not have an inhibitory effect on nuclear Ca^{2+}-dependent endonuclease which is involved in Ca^{2+}-signalling.

Other Roles of RGPR-P117 in Cell Regulation

RGPR-p117 has been found to localize in the plasma membarnes, nucleus, mitochondria, microsomes (endplasmic reticulum) and cytoplasm using Western blot analysis for HA-RGPR-p117, when subcellualr fractions were prepared from the homogenate of NRK52E cells transfected with HA-RGPR-p117/phCMV2 [21]. This finding suggests that RGPR-p117 also participates in the regulation of other cell functions. After discovery of RGPR-p117, this protein was renamed as Sec16B, which is involved in the endoplasmic reticulum (ER) export [29]. Sec16 is a large peripheral ER membrane protein that functions in generating COPII transport vesicles and in clustering COPII components at transitional ER sites. Sec16 interacts with multiple COPII components. Mammalian cells contain two distinct Sec16 homologues which are termed as the larger protein Sec16L and the smaller Sec16S (Sec16B) [29]. These proteins localize to transitional ER sites [29]. Human Sec16B, which is encoded by a gene on chromosome 1, is higher homology to RGPR-p117, except for a few amino acid substitutions, suggesting that RGPR-p117 plays a role in ER export in the cells [29]. From this, RGPR-p117 was renamed as Sec16B [29]. This was not suitable with many scientific findings concerning the role of RGPR-p117 in cell regulation. RGPR-p117 may not be Sec16B. RGPR-p117, which was originally discovered as a novel transcription factor, has been demonstrated to have many characterizations as transcription factor. Moreover, there is growing evidence that RGPR-p117 plays many roles in cell regulation.

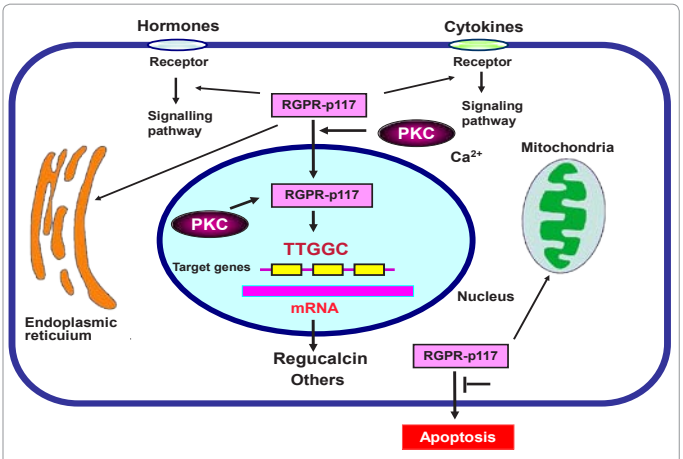

Figure 1: Role of RGPR-p117 in cell regulation. RGPR-p117 localizes into the nucleus, which is mediated through protein kinase C (PKC)- dependent signaling process, and it regulates gene expression that is related to the TTGGC motif. Also, RGPR-p117 has suppressive effects on apoptosis, which is induced through various signaling stimulations. In addition, RGPR-p117 binds to the plasma membranes, mitochondria and endoplasmic reticulum in cells and may regulate their organellar functions. RGPR-p117 may have effects on cytoplasmic enzyme activity.

Prospect

The roles of RGPR-p117 in cell function are summarized in figure 1. In addition, the effect of RGPR-p117, which is a transcription factor, in cell regulation may be partly mediated through RGPR-p117-regulated gene expression for various proteins including regucalcin which plays a multifunctional role in cell regulation [3,4]. Pathophysiological role of RGPR-p117 remains to be elucidated. The clone MGC: 17455 (DDBJ/EMBL/GenBank accession number BC009106) is derived from human placental choriocarcinoma which is a splicing variant of human RGPR-p117 gene [1]. This incomplete splicing for the human RGPR-p117 gene may be involved in carcinogenesis in the placenta. In addition, the development of pharmaceutical tool, which targets RGPR-p117 molecule, may lead to elucidation of its role in cell regulation.

Author Contribution and Disclosures

The author contributed to the design and conduct of the study, collection, analysis, and interpretation of data, and manuscript writing. Author has no conflicts of interest.

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