Regucalcin, cell signalling-related protein: its multifunctional role in kidney cell regulation

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Abstract

Introduction
Regucalcin was discovered in 1978 as a calcium-binding protein that does not contain EF-hand motif of calcium-binding domain, which differs from calmodulin and other calcium-related proteins. The regucalcin gene is localized on the X chromosome. Regucalcin is greatly pronounced in hepatocytes and kidney proximal tubular cells. This review provides recent information concerning the role of regucalcin in the regulation of kidney cell function.

Discussion
Regucalcin has been demonstrated to play a physiological role in the regulation of Ca2+ homeostasis in the kidney proximal tubular epithelial cells, which participate in transcellular transport to reabsorption of calcium from filtrated urine calcium. Regucalcin also regulates various enzyme activities including Ca2+-dependent protein kinases, protein phosphatases, nitric oxide synthase and others, which are involved in intracellular signalling pathways. Regucalcin is localized to the nucleus and regulates the gene expressions of proteins, which are related to mineral transport-related proteins, Smad 2, the nuclear factor-kappa B, cell proliferation and apoptosis-related proteins in the kidney tubular epithelial cells.

Conclusion
This review proposes that regucalcin plays a pivotal role as a multi-functional protein in the regulation of kidney proximal tubular epithelial cells. It reveals a suppressive effect on cell proliferation and apoptotic death, through signalling stimulators and hypertensive state or developing drug-induced kidney disorders.

Introduction
Calcium signals mediate many intracellular responses, which are amplified through calmodulin and protein kinase Cα-3. Regucalcin (RGN) was discovered in 1978 as a calcium-binding protein that does not contain EF-hand motif of calcium-binding domain, differing from calmodulin and other related proteins. The name RGN is proposed for this calcium-binding protein, which suppresses various Ca2+- or Ca2+/calmodulin-dependent enzyme activations5-7. RGN and its gene (rgn) are identified in over 15 species consisting of RGN family and is highly conserved in vertebrate species throughout evolution8-10. The rgn gene is localized on the X chromosome11,12. The organization of the rgn gene consists of seven exons and six introns13. Various transcription factors (including AP-1, NF1-A1, RGRF-P17, β-catenin, SP1 and others) are identified as the enhancer and suppressor for rgn gene expression14-19. mRNA expression of rgn and its protein content are pronounced in the liver and kidney cortex of rats14-20, and rgn expression is regulated through various hormonal stimulations and physiological states9.

The role of RGN in cellular function is focused in the liver in detail21-23. RGN plays a role in the maintenance of intracellular calcium ion (Ca2+) homeostasis24, depression of calcium signalling from the cytoplasm to the nucleus in proliferating cells25 and regulation of nuclear functions26,27. Overexpression of endogenous RGN in liver cells suppresses cell proliferation28 and apoptotic cell death29, which is induced by various signalling factors. RGN has been proposed to play a pivotal role as a suppressor protein in the regulation of cellular function in maintaining cell homeostasis that is mediated through various signalling pathways30,31.

RGN is predominantly expressed in the liver and kidney cortex including kidney proximal tubular epithelial cells20. The role of RGN is elucidated in liver cells21,22. This review outlines recent advances made concerning the regulation of rgn expression and the role of RGN in the kidney proximal tubular epithelial cells.

Discussion
The author has referenced some of its own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. Animal care was in accordance with the institutional guidelines.

Hormonal regulation of rgn expression
RGN is expressed in the kidney tissues of rats14-20. The concentration of RGN in rat kidney tissues is in the range of 1.74–3.50 × 107 M in male or female rats measured by using enzyme-linked immunoadsorbent-sorbent assay20. This level is not lowered with aging20.

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Regulation of rgn expression in vivo

The expression of RGN mRNA is predominant in the kidney cortex but not in the medulla of rats. The kidney cortex constitutes nephrons including glomerulo and tubulo. RGN mRNA expression is enhanced in the kidney cortex after a single intraperitoneal administration of calcium chloride in rats. The administration of calcium (100 and 150 mg/kg body weight) causes a remarkable increase in serum calcium concentration and a significant elevation of calcium content in the kidney cortex at 20 min after calcium administration. The expression of RGN mRNA in the kidney cortex is increased at 60 min after calcium administration. These findings suggest that RGN mRNA expression is related to calcium that is increased in the kidney cortex after calcium administration. RGN mRNA expression is also enhanced after calcium administration in thyroparathyroidectomized rats with deficiency of calcium-regulation hormones (calcitonin and parathyroid hormone), which are secreted from thyroid-parathyroid glands. Calcium-regulating hormones regulate calcium reabsorption in the kidney proximal tubular cells. The stimulating effect of calcium administration on kidney RGN mRNA expression may be independent on calcium-regulating hormones.

The calcium administration-stimulated RGN mRNA expression in the kidney cortex of rats in vivo is completely blocked after the treatment of trifluoperazine (TFP), an inhibitor of Ca²⁺/calmodulin, suggesting that the expression is mediated through Ca²⁺/calmodulin that is involved in the activation of protein kinases. The nuclear proteins in the kidney cortex have been shown to specifically bind to the 5′-flanking region of rat rgn, and its binding activity is partly mediated through the Ca²⁺/calmodulin-signalling pathway. This specific nuclear factor binds to the NF1 consensus motif TTTGCG(N)₆ CC in the promoter region of rgn in the rat kidney cortex. NF1 binding motif in the promoter region of rgn may be partly involved in the transcriptional regulation of rgn expression in the kidney cortex cells.

Steroid hormones have also been shown to regulate RGN mRNA expression in the kidney cortex of rats. RGN mRNA expression is stimulated after a single subcutaneous administration of dexamethasone (1 mg/kg body weight) in rats, while it is suppressed after a single subcutaneous administration of aldosterone (25–100 μg/kg) or oestrogen (0.5–2 mg/kg). The administration of hydrocortisone (5–30 mg/kg) does not have an effect on RGN mRNA expression in the kidney cortex, rgn may have response elements for receptors of glucocorticoid, aldosterone or oestrogen. The dexamethasone-induced increase in RGN mRNA expression in the kidney cortex is completely suppressed after simultaneous administration of cycloheximide, an inhibitor of protein synthesis. This finding may be partly based on newly synthesized DNA binding proteins (steroid receptors) in the kidney cortex.

RGN mRNA expression in the kidney cortex of rats has been shown to attenuate in a pathophysiological state. RGN mRNA expression suppression in the kidney cortex of adrenalectomized (ADX) rats may deplete endogenous steroid hormones, which are secreted from adrenal glands. However, the treatment of dexamethasone in ADX rats does not restore the ADX-induced suppression of RGN mRNA expression in the kidney cortex. Adrenal glands, however, may participate in the regulation of RGN mRNA expression in the kidney cortex of rats.

RGN mRNA expression has been shown to be suppressed in the kidney cortex of normal or ADX rats upon oral intake of saline for 7 days, which may cause a hypertensive condition. This finding may be partly based on newly synthesized DNA binding proteins (steroid receptors) in the kidney cortex of rats. The intake of saline has been shown to cause an alteration in calcium metabolism, which is related to calcium transport in the kidney cortex of rats. Saline intake for 7 days in rats causes a significant increase in serum calcium concentration and an elevation of Ca²⁺-ATPase activity in the basolateral membranes of the kidney cortex and a corresponding increase in renal cortex calcium content. Saline intake-induced hypercalcemia may partly result from the tubular reabsorption of urinary calcium. Blood urea nitrogen (BUN) concentration was increased in rats with saline intake for 7 days, suggesting that saline intake causes renal disorder. Interestingly, RGN mRNA expression is suppressed in the kidney cortex of spontaneous hypertensive rats (SHR). Saline intake, which may generate a hypertensive condition, causes a suppression of RGN mRNA expression in the kidney cortex of rats. The suppressed rgn expression may have a role in the development of renal hypertension.

Various drugs are known to have a side effect of causing renal damage. Depending on the chemical, the mechanism of nephrotoxicity may involve direct interference with tubular or mitochondrial transport processes, covalent modification of critical cellular constituents or generation of free radicals. Cisplatin, a nephrotoxic antitumour drug, or cephaloridine, a nephrotoxic cephalosporin antibiotic, is known to change the thiol status in the renal cortex before the development of significant morphological changes. RGN mRNA expression in the kidney cortex is markedly suppressed at 24 hours after a single intraperitoneal administration of cisplatin (2.5–10 mg/kg body weight) or cephaloridine (250–1000 mg/kg body weight).
Hormonal regulation of *rgn* expression was examined using cloned normal rat kidney proximal tubular epithelial NRK52E cells in vitro. Parathyroid hormone (PTH), 1,25-dihydroxyvitamin D$_3$ or calcitonin play a role in the regulation of calcium transport in the kidney proximal tubular epithelial cells. Among these hormones, PTH (10 and 100 nM) has been found to stimulate RGN mRNA expression and its protein level in NRK52E cells. PTH has a stimulatory effect on the reabsorption of calcium in the kidney proximal tubule.

RGN plays a physiological role as a key molecule in the reabsorption of calcium in the kidney proximal tubule. The expression of RGN mRNA in NRK52E cells is enhanced by aldosterone (10 and 100 nM), which has a stimulatory effect on the reabsorption of sodium in the kidney proximal tubule. The ATP-dependent Ca$^{2+}$ pump and Na$^+$/Ca$^{2+}$ exchange system are constituted in the basolateral membranes of the rat kidney cortex. It is possible that the effect of aldosterone in the kidney proximal tubule is partly mediated through RGN expression. In addition, RGN mRNA in NRK52E cells is enhanced after culture with dexamethasone (10 nmol) and the effect of aldosterone or dexamethasone in the kidney proximal tubule is partly mediated through RGN.

RGN mRNA expression has been found to be enhanced by dibutyryl cyclic adenosine monophosphate (DcAMP) (10 and 100 mM) or phorbol-12-myristate-13-acetate (PMA; 1 μM) in NRK52E cells. PMA has a role as an activator of protein kinase C. DcAMP may have a role as a second messenger. The expression of RGN mRNA in NRK52E cells may be partly mediated through signalling pathways that are related to cAMP or protein kinase C. The action of PTH has been known to be mediated by cAMP or inositol 1,4,5-triphosphate (IP$_3$)-released Ca$^{2+}$ and protein kinase C in the cells. The effect of PTH in stimulating RGN mRNA expression in NRK52E cells may be mediated by cAMP and/or Ca$^{2+}$-dependent protein kinase C in NRK52E cells.

Dibucaine is an antagonist of Ca$^{2+}$/calmodulin-dependent protein kinase. PD98059 is an inhibitor of the extracellular signal-related kinase (ERK) pathway. RGN mRNA expression is not altered in the presence of dibucaine or PD98059 in NRK52E cells, suggesting that the expression is not mediated through Ca$^{2+}$/calmodulin-dependent protein kinase or mitosis-activated protein (MAP) kinase, which is related to the ERK pathway. RGN mRNA expression in NRK52E cells has been found to be suppressed after culture with staurosporine, an inhibitor of protein kinase C. Meanwhile, RGN mRNA expression is markedly enhanced after culture with PMA, an activator of protein kinase C. This finding further supports the view that RGN mRNA expression is partly mediated through cell signalling that is related to protein kinase C in NRK52E cells. Culture with vanadate, which is an inhibitor of protein tyrosine phosphatase, did not cause a significant alteration in the RGN mRNA expression in NRK52E cells, suggesting that the expression is not involved in cell signalling that is related to protein tyrosine phosphatase.

Interestingly, RGN mRNA expression is suppressed after culture with tumour necrosis factor-α (TNF-α) or transforming growth factor-β (TGF-β) in NRK52E cells. These factors, which repress RGN mRNA expression, have been poorly understood.

Transcription factor NF1-A1 and RGPR-p117 are involved in the enhancement of *rgn* expression. NF1-A1 and RGPR-p117 are identified as hepatic nuclear factors that bind to the TTGGC sequence of the rat *rgn* promoter region using a yeast one-hybrid system. Over expression of RGPR-p117 has been shown to enhance the rat *rgn* promoter activity that is involved in the TTGGC sequence in NRK52E cells. NF1-A1 enhances the *rgn* promoter activity, which is related to the TTGGC motif, and this enhancement is not revealed in the mutant with deletion of the TTGGC(N)$_6$ sequence in NRK52E cells. NF1-A1 has been shown to localize in the nuclei of NRK52E cells and increase the *rgn* promoter activity in the cells. This increase is enhanced after culture with Bay K 8644, an agonist of calcium entry into cells, or PMA, an activator of protein kinase C. Ca$^{2+}$-dependent protein kinases

may be involved in enhancing the rgn promoter activity in NRK52E cells. In addition, increases in the rgn promoter activity in NRK52E cells is blocked after culture with dibucaine, strophobine, PD98059, vanadate or okadaic acid, which are inhibitors of various Ca\(^{2+}\)-dependent protein kinases and protein phosphatases\(^{39}\). The rgn promoter activity in NRK52E cells may be enhanced through various intra-cellular signalling factors including Ca\(^{2+}\)-dependent protein kinases, MAP kinase and protein phosphatases.

RGRP-p117 increases the rat rgn promoter activity in NRK52E cells\(^{57,58}\). Co-transfection with NF1-A1 and RGRP-p117 does not significantly enhance the RGRP-p117-increased rgn promoter activity in NRK52E cells\(^7\). NF1-A1 or RGRP-p117 is localized in the nuclei of NRK52E cells. These transcription factors may independently regulate in the enhancement of the rat rgn promoter activity in NRK52E cells\(^7\). Various signalling factors are involved in the enhancement of the rat rgn promoter activity in NRK52E cells overexpressing NF1-A1 or RGRP-p117. This enhancement seems to be mediated through protein phosphorylation and dephosphorylation in NRK52E cells. NF1-A1 or RGRP-p117, however, acts independently in the enhancement of the rat rgn promoter activity in NRK52E cells. NF1-A1 or RGRP-p117 plays a role as a transcription factor (enhancer) in the rat rgn promoter activity.

**RGN regulates intracellular calcium homeostasis**

Kidneys play a physiological role in regulating calcium homeostasis in blood by reabsorption of urinary calcium\(^{33,34,47}\). Renal cortex cells constituting the proximal tubular epithelial cells may play a role in this. RGN mRNA is expressed in the kidney cortex but not in the medulla of rats and its expression is stimulated by calcium administration in vivo\(^{42}\). RGN may play a role in the regulation of calcium transport in the renal proximal tubular epithelial cells.

Active Ca\(^{2+}\) reabsorption is transcellularly transported and Ca\(^{2+}\) pumps in the basolateral membranes have to be involved to overcome the step of energy barriers at the peritubular cell side\(^47\). The regulation of intracellular Ca\(^{2+}\) homeostasis is important in the promotion of intracellular Ca\(^{2+}\) transport. The low cytoplasmic Ca\(^{2+}\) concentration of living cells is maintained through energy-requiring pumps. These pumps either remove Ca\(^{2+}\) to the extracellular space by transporting it across the plasma membrane or accumulate it inside intracellular organelles such as the mitochondria and endoplasmic reticulum. Intracellular Ca\(^{2+}\) homeostasis is regulated through plasma membrane (Ca\(^{2+}\)-Mg\(^{2+}\)))-adenosine 5’-triphosphatase (ATPase), microsomal Ca\(^{2+}\)-ATPase, mitochondrial Ca\(^{2+}\) uptake and nuclear Ca\(^{2+}\) transport in the cells. RGN has been shown to regulate Ca\(^{2+}\)-transporting systems in the renal cortex cells.

The Ca\(^{2+}\)-ATPase system exceeds the capacity of the Na\(^+\)/Ca\(^{2+}\) exchanger and plays a primary role in Ca\(^{2+}\) homeostasis of rat kidney cortex cells\(^7\). RGN has been found to play a role as an activator of the ATP-dependent Ca\(^{2+}\) pumps (Ca\(^{2+}\)-ATPase) in the basolateral membranes isolated from the rat kidney cortex; RGN (100 and 1000 nM) increased Ca\(^{2+}\)-ATPase activity and stimulated Ca\(^{2+}\) uptake by the basolateral membranes in vitro\(^{46}\). This effect of RGN is not seen in the presence of digitonin, which can solubilize the lipids of the plasma membranes in an enzyme reaction mixture\(^46\). Also, the effect of RGN on Ca\(^{2+}\) pump enzyme activity is completely inhibited in the presence of vanadate, an inhibitor of phosphorylation of ATPase, or N-ethylmaleimide, a SH-group modifying reagent. RGN may increase Ca\(^{2+}\)-ATPase activity by binding to the SH-group of active sites of the enzyme and stimulating the phosphorylation of the enzyme in the microsomes of the rat kidney cortex\(^{60}\). The kidney cortex microsomal Ca\(^{2+}\)-ATPase activity and ATP-dependent Ca\(^{2+}\) uptake is inhibited by DcAMP or IP\(^3\). Calmodulin, a modulator of Ca\(^{2+}\) signalling, had a stimulating effect on microsomal Ca\(^{2+}\)-ATPase activity, although the stimulating effect of calmodulin is lower than that of RGN. Both proteins may be important as an activator in the microsomal ATP-dependent Ca\(^{2+}\) sequestration\(^{60}\).

An ATP-dependent Ca\(^{2+}\) uptake system (Ca\(^{2+}\)uniporter) exists in the mitochondria of the kidney cortex of rats\(^3\). RGN (50–250 nM) has been shown to stimulate Ca\(^{2+}\)-ATPase-related Ca\(^{2+}\)uniporter activity in the mitochondria\(^4\). The effect of RGN is completely blocked by ruthenium red or lanthanum chloride, which is a specific inhibitor of Ca\(^{2+}\) uniporter in the mitochondria\(^4\), suggesting that RGN stimulates Ca\(^{2+}\)-ATPase-related Ca\(^{2+}\)uniporter activity in renal cortex mitochondria. RGN may bind to the membranous lipids of renal cortex mitochondria and act on the
SH-groups, which are active sites of Ca\textsuperscript{2+}-ATPase\textsuperscript{45}. Calmodulin or DcAMP does not modulate the effect of RGN in increasing mitochondrial Ca\textsuperscript{2+}-ATPase activity\textsuperscript{41}.

The reabsorption of urinary calcium is promoted through transcellular Ca\textsuperscript{2+} transport in the renal proximal tubular epithelial cells\textsuperscript{45}. RGN stimulates Ca\textsuperscript{2+} transport (efflux) across the basolateral membranes of the renal cortex\textsuperscript{46}, the microsomal ATP-dependent Ca\textsuperscript{2+} sequestration\textsuperscript{47} and the mitochondrial ATP-dependent Ca\textsuperscript{2+} uptake to maintain intracellular Ca\textsuperscript{2+} concentration\textsuperscript{48} in the rat renal cortex as shown in Figure 1. Thus, RGN may play a physiological role in the regulation of intracellular Ca\textsuperscript{2+} homeostasis in the renal proximal tubular epithelial cells due to activating ATP-dependent Ca\textsuperscript{2+}-transport systems in the basolateral membranes, microsomes and mitochondria. RGN may promote Ca\textsuperscript{2+} reabsorption, which is based on ATP-dependent transcellular transport of Ca\textsuperscript{2+}, in the proximal tubular epithelial cells of the nephron tubule of the kidney cortex. Moreover, RGN may play a physiological role in the regulation of calcium homeostasis in the blood through reabsorption of urinary calcium in the kidney.

**RGN regulates signalling pathway-related enzyme activity**

Multifunctional Ca\textsuperscript{2+}/calmodulin-dependent protein kinases play an important role in the response of many cells for a calcium signal\textsuperscript{42,43}. RGN (0.01–1 μM) has been found to have an inhibitory effect on the activation of Ca\textsuperscript{2+}/calmodulin-dependent protein kinase in the cytoplasm of the rat kidney cortex in vitro\textsuperscript{44}. The effect of RGN in inhibiting Ca\textsuperscript{2+}/calmodulin-dependent protein kinase activity in the cytoplasm of the renal cortex is also seen at the greater concentration of added calcium chloride (100–1000 μM) in an enzyme reaction mixture\textsuperscript{44}, suggesting that the inhibitory effect of RGN on the enzyme activity does not mainly result from the binding of Ca\textsuperscript{2+} by RGN. Moreover, the inhibitory effect of RGN is seen in the presence of higher concentrations of calmodulin (4–20 μg/ml) in an enzyme reaction mixture\textsuperscript{44}. RGN may have a direct inhibitory effect on Ca\textsuperscript{2+}/calmodulin-dependent protein kinase in renal cortex cytosol. It is possible that RGN has a partial inhibitory effect on the binding of Ca\textsuperscript{2+}-calmodulin to the enzyme.

Protein kinase C is a diacylglycerol-activated Ca\textsuperscript{2+}- and phospholipids-dependent protein kinase that is widely distributed in the body\textsuperscript{45}. Protein kinase C is capable of phosphorylating many proteins. Protein kinase C is an important enzyme, which is related to Ca\textsuperscript{2+} signalling in many cell types. RGN has been found to inhibit protein kinase C activity in the cytoplasm of the rat kidney cortex in vitro\textsuperscript{46}. The inhibitory effect of RGN (0.01–1 μM) on protein kinase C activity in the presence of Ca\textsuperscript{2+}, phosphatidylserine and dioctanoylglycerol is not seen in a reaction mixture without Ca\textsuperscript{2+} addition\textsuperscript{47}. Moreover, RGN has an inhibitory effect on protein kinase C activity raised by addition of diacylglycerol or 12-myristate 13-acetate (PMA), which can directly activate the enzyme, in the presence of both Ca\textsuperscript{2+} and phosphatidylserine\textsuperscript{48}. RGN may bind to protein kinase C, and the RGN binding-induced conformational alteration of the enzyme may inhibit the binding of Ca\textsuperscript{2+} to the kinase.

Thus, RGN may play a pivotal role as a suppressor protein in the regulation of Ca\textsuperscript{2+} signalling-dependent cellular functions, which are mediated through Ca\textsuperscript{2+}/calmodulin-dependent protein kinase and protein kinase C, in the kidney cortex cells.

Protein phosphorylation-dephosphorylation is a universal mechanism by which numerous cellular events are regulated\textsuperscript{49}. It has become apparent

![Figure 1](image)  
*Figure 1:* RGN promotes transcellular transport of urinary Ca\textsuperscript{2+} in the kidney proximal tubular epithelial cells. The entry of urinary Ca\textsuperscript{2+} in the cells stimulates the expression of RGN mRNA and induces an increase in RGN. RGN activates Ca\textsuperscript{2+} pump enzyme (Ca\textsuperscript{2+}-ATPase) in the basolateral membranes, endoplasmic reticulum (microsomes) and mitochondria. RGN plays a pivotal role in maintaining intracellular Ca\textsuperscript{2+} homeostasis. RGN may play a physiological role in the promotion of reabsorption of urinary Ca\textsuperscript{2+}.

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that there may exist many phosphatases that, like the kinases, are elaborately and rigorously controlled. Protein phosphatase plays an important role in intracellular signal transduction due to hormonal stimulation. Calcineurin, a calmodulin-binding protein, possesses a Ca\(^{2+}\)-dependent and calmodulin-stimulated protein phosphatase activity. Calcineurin is a protein serine/threonine phosphatase. RGN (0.01–1 μM) has been found to have an inhibitory effect on Ca\(^{2+}\)/calmodulin-dependent phosphatase (calcineurin) activity in rat renal cortex cytoplasm in vitro. The inhibitory effect of RGN on Ca\(^{2+}\)/calmodulin-dependent protein phosphatase activity in the renal cortex cytoplasm is not weakened by the addition of higher concentrations of calcium chloride. In addition, RGN does not have an effect on protein phosphatase activity in the presence of Ca\(^{2+}\)-chelator without calmodulin, suggesting that the effect of RGN may not result from a direct action on the enzyme. RGN can bind to calmodulin in an experiment using calmodulin-agarose beads in vitro. The inhibitory effect of RGN on Ca\(^{2+}\)/calmodulin-dependent protein phosphatase activity in the renal cortex cytosol may be partly based on its binding to calmodulin. It cannot exclude, however, that RGN directly binds the enzyme.

RGN (50–250 nM) has also been shown to have an inhibitory effect on Ca\(^{2+}\)/calmodulin-independent protein tyrosine phosphatase activity in rat renal cortex cytosol, although the addition of RGN into an enzyme reaction mixture does not have an inhibitory effect on protein phosphatase activity toward three phosphoamino acids in the renal cortex cytosol, suggesting that the endogenous RGN, which is present in the cortex cytoplasm, has a suppressive effect on the activity of various protein phosphatases in the kidney cortex cells. Protein phosphatase activity is present in the nuclei of kidney cortex cells. Ca\(^{2+}\)/calmodulin-dependent protein phosphatase (calcineurin) and protein tyrosine phosphatase may be present in the nuclei of rat kidney cortex cells. RGN is localized in the nucleus of the rat kidney cortex. RGN (25–100 nM) has an inhibitory effect on protein tyrosine phosphatase and protein serine/threonine phosphatase activities in the nucleus of renal cortex cells. The endogenous RGN in the nucleus of kidney cortex cells suppresses various protein phosphatases, which are present in the nucleus, using RGN monoclonal antibody.

RGN mRNA levels in the kidney cortex are decreased after saline intake for 7 days in rats. RGN in the cytoplasm and nucleus of the kidney cortex is decreased after intake of saline for 7 days in rats. The intake of saline causes a remarkable decrease in protein phosphatase activity in the cytoplasm and nuclei. In addition, the effect of anti-RGN monoclonal antibody in increasing protein phosphatase activity in the cytoplasm and nuclei is weakened after saline intake, supporting the involvement of endogenous RGN in the regulation of protein phosphatase activity. A single intraperitoneal administration of calcium (25–100 mg/kg body weight) in rats has been found to produce an increase in calcium content and a corresponding elevation of RGN in the cytoplasm and nucleus of the kidney cortex. Calcium administration causes an increase in protein phosphatase activity in the cytoplasm and nucleus of the kidney cortex, suggesting that the increase in the enzyme activity is partly mediated through Ca\(^{2+}\) signalling, which results from the augmentation of renal calcium content. The presence of anti-RGN monoclonal antibody in the enzyme reaction mixture causes an increase in protein phosphatase in the cytoplasm and nucleus of the normal rat kidney cortex. This effect is significantly enhanced in the cytoplasm and nucleus of the kidney cortex in calcium-administered rats. This finding demonstrates that the calcium administration-induced increase in endogenous RGN suppresses the enhancement of protein phosphatase activity in the cytoplasm and nucleus of the kidney cortex in calcium-administered rats.

Dephosphorylation of many phosphorylated proteins is regulated by protein phosphatase in various cells. Protein phosphatase may be implicated in transcriptional regulation in the nucleus of cells. RGN can inhibit protein phosphatase activity in the nucleus of the rat kidney cortex, suggesting that RGN plays an important role in the regulation of nuclear signalling, which is related to the gene expression in the nucleus of renal cortex cells.

cAMP is generated through the activation of the plasma membrane adenylate cyclase due to hormonal stimulation in many cell types and activates cAMP-dependent protein kinase, which plays an important role in cAMP signalling pathway. cAMP is degraded by cAMP phosphodiesterase, which is activated by Ca\(^{2+}\)/calmodulin. RGN inhibits Ca\(^{2+}\)/calmodulin-dependent cAMP phosphodiesterase activity in the cytosol of the rat renal cortex. RGN may play a role in the regulation of both cAMP-dependent and Ca\(^{2+}\)-dependent signalling pathways that are modulated through hormonal stimulation in the renal cortex cells.

Nitric oxide (NO) acts as a messenger or modulator molecule in many biological systems. NO, which has an unpaired electron that reacts with proteins and targets primarily through their thiol or heme groups,
acts as a messenger or modulator molecule in many biological systems. NO is produced from L-arginine with L-citrulline as a co-product in a reaction catalysed by NO synthase that requires Ca²⁺/calmodulin. RGN has been shown to inhibit NO synthase activity in the cytoplasm of the rat liver. RGN also has an inhibitory effect on Ca²⁺/calmodulin-dependent NO synthase activity in the rat renal cortex cytoplasm, suggesting that RGN has a suppressive effect on overproduction of NO in the cells. The endogenous RGN may depress NO synthase activity in the kidney cortex cytoplasm of RGN transgenic rats as compared with that of wild-type rats. The effect of calcium chloride (10 μM) in increasing NO synthase activity in the kidney cortex cytosol of wild-type rats is weakened in that of RGN transgenic rats. The presence of anti-RGN monoclonal antibody (25 or 50 ng/ml) in the reaction mixture caused a significant increase in NO synthase activity. This increase completely disappeared after the addition of RGN (100 nM). Endogenous RGN has a suppressive effect on NO synthase activity in the kidney cortex cytoplasm of rats.

RGN has been demonstrated to have a suppressive effect on Ca²⁺/calmodulin-dependent NO synthase activity in the kidney cortex. NO acts as a messenger or modulator in kidney cortex cells. RGN may play a role as a suppressor protein in NO production in the kidney cells and may regulate many cellular events that are involved in NO signalling. NO production may be stimulated through Ca²⁺ signalling due to hormonal stimulation in kidney cortex cells. RGN may have a suppressive effect on overproduction of NO in kidney cortex cells. As mentioned above, RGN may play a role as a suppressor for activation of many enzymes which are related to signalling pathways that are mediated through calcium, cAMP, NO, Ca²⁺-dependent protein kinases and protein phosphatases in the kidney cortex cells. The mechanism by which RGN has an inhibitory effect on Ca²⁺/calmodulin-dependent enzyme activity may be partly based on its binding to Ca²⁺/calmodulin and/or enzyme. RGN has been demonstrated to bind calmodulin in analysis with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using calmodulin-agarose beads. It is possible that RGN directly binds to Ca²⁺ and/or enzyme to reveal the inhibitory effect on enzyme activity.

Interestingly, RGN has been found to stimulate proteolytic activity in the cytoplasm of the rat kidney. RGN uniquely activates thiol proteases independent of Ca²⁺ in the cytoplasm of the rat kidney cortex, although it does not have an effect on serine proteases and metalloproteases. The effect of RGN (10–250 nM) in increasing proteolytic activity in the cytoplasm of the rat kidney cortex is seen in the presence of ethyleneglycol bis (2 aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), a chelator of Ca²⁺, although Ca²⁺ addition-increased proteolytic activity was completely abolished by the addition of chelator, suggesting that RGN increases proteolytic activity independent of Ca²⁺ in the kidney cortex cytoplasm. RGN directly acts on the SH-groups of protease in the kidney cortex cytoplasm. The molecular mechanism by which RGN activates thiol proteases (calpains) in the kidney cortex cytoplasm remains to be elucidated. RGN activates protease at concentrations of 10–250 nM in vitro experiments. The concentration of RGN present in rat kidney tissues is about 5.3 μM. RGN may play a physiological role in the activation of thiol proteases in renal cortex cells.

Calpains are thiol (SH) proteases. Calpains are ubiquitous, non-lysosomal, calcium-dependent proteases that may play important roles in Ca²⁺-mediated intracellular processes. The ability of calpains to alter limited proteolysis, activity or function of numerous cytoskeletal proteins, protein kinases, receptors and transcription factors suggests its involvement in various Ca²⁺-regulated cellular functions. RGN increases the activity of thiol proteases including calpains in the cytoplasm of the rat kidney cortex. RGN may play a pivotal role in the regulation of cellular functions related to Ca²⁺ that is mediated through thiol proteases. Presumably, RGN plays a role in the regulation of signal transduction that is involved in proteases.

The stimulatory effect of RGN on proteolytic activity is impaired in the kidney cortex cytoplasm of rats with the intake of saline for 7 days, which suppresses RGN mRNA expression in the kidney cortex. Proteases, which are activated by RGN, may be reduced in the kidney cortex cytoplasm of saline-administered rats. In addition, the saline administration-induced decrease in proteolytic activity of rat renal cortex cytoplasm is partly involved in the suppressed RGN expression.

The physiological role of RGN in the activation of thiol proteases in kidney cortex cells remains to be elucidated. It is possible, however, that RGN plays a role in the regulation of the degradation of proteins that are involved in the regulation of signalling pathways. In addition, RGN may play a part in the regulation of protein turnover in kidney cortex cells.

RGN regulates nuclear function

Nuclear localization of RGN

RGN has been found to localize in the nucleus of HA-regucalcin/phCMV2-transfected NRK52E cells using immunocytochemical analysis. The nuclear localization of RGN was enhanced after culture with BTH, Bay K 8644 or PMA. This enhancement was remarkable after culture with PMA. PMA-enhanced RGN expression is suppressed by staurosporine, an inhibitor of protein kinase C. RGN, which is enhanced through Ca²⁺-signalling factors including protein kinase C, is localized in the nucleus of NRK52E cells. The results of Western blot analysis demonstrate that the
nuclear RGN levels are markedly increased after culture with PMA. This increase is depressed in the presence of staurosporine, an inhibitor of protein kinase C. Thus, the results with Western blot analysis support the observation with immunocytochemical analysis for the nuclear localization of RGN in NRK52E cells.

The nuclear localization of RGN in stable RGN/pCXN2-transfected cells (transfectants) is markedly localized as compared with that of wild-type cells. This increase is not seen in the transfectants cultured with staurosporine. These observations support the view that the localization of RGN into the nuclei is enhanced through Ca²⁺ signalling related to protein kinase C in NRK52E cells.

The expression of RGN mRNA in NRK52E cells may be partly mediated through signalling pathways that are related to cAMP or protein kinase C. The action of PTH is mediated through cAMP or IP₃-released Ca²⁺ and protein kinase C in cells. The effect of PTH in stimulating the nuclear localization of RGN in NRK52E cells may be mediated through cAMP and/or Ca²⁺-dependent protein kinase C in NRK52E cells. In addition, PMA, an activator of protein kinase C, has been shown to enhance markedly the nuclear localization of RGN in NRK52E cells. Thus, protein kinase C may play a pivotal role in the enhancement of both RGN mRNA expression and the nuclear localization of RGN protein in NRK52E cells. The nuclear localization of RGN, which is enhanced through hormonal signalling, may play a role in the regulation of the expression of many genes in the nuclei.

RGN suppresses nuclear DNA synthesis

RGN has been shown to have an inhibitory effect on DNA synthesis activity in the nuclei of normal rat liver. Ca²⁺ is present in the liver nucleus. The nuclear DNA synthesis activity is increased in the presence of EGTA, a chelator of Ca²⁺, in a reaction mixture in vitro, suggesting an inhibitory effect of the nuclear endogenous Ca²⁺. RGN (0.1–0.5 μM) has a suppressive effect on DNA synthesis activity in the nuclei isolated from the rat renal cortex in vitro. The effect of RGN is also seen in the presence of calcium chloride (50 μM) in the reaction mixture and is enhanced in the presence of EGTA (1 mM). RGN may have an inhibitory effect on DNA synthesis in the nucleus of the renal cortex through a mechanism that is not related to Ca²⁺. The presence of anti-RGN monoclonal antibody (10–50 ng/ml) in the reaction mixture caused a significant increase in the nuclear DNA synthesis activity. This increase is completely abolished in the presence of RGN (0.5 μM). The nuclear endogenous RGN has been found to have a suppressive effect on DNA synthesis in the nuclei of the rat renal cortex.

The suppressive effect of RGN on nuclear DNA synthesis is also observed in the presence of various inhibitors (staurosporine, TFP or okadaic acid) for protein kinases and protein phosphatases, which are involved in the regulation of the nuclear functions including cell proliferation and gene expression, in the reaction mixture. These results suggest that the effect of RGN in decreasing the nuclear DNA synthesis activity is not related to protein kinases and protein phosphatases in the renal cortex nucleus. RGN may directly bind to DNA and inhibit DNA synthesis. RGN may play a role as a suppressor in DNA synthesis activity in the renal cortex nucleus.

Role of RGN in proximal tubular epithelial cells

Proximal tubular epithelial cells are predominantly present in the kidney cortex. NRK52E cells are cloned from the normal rat kidney cortex. RGN has been shown to express in proximal tubular epithelial NRK52E cells in vitro. The role of RGN in the regulation of kidney cell function is examined using NRK52E cells.

Overexpression of endogenous RGN suppresses cell proliferation

RGN/pCXN2-transfected cells (transfectants) for NRK52E cells, which stably overexpress RGN, have been generated to determine the role of endogenous RGN in the regulation of cell function. The RGN content of RGN/pCXN2-transfected cells was about 21-fold as compared with that of the parental wild-type NRK52E cells. Overexpression of endogenous RGN has been found to have a role in the regulation of the proliferation of NRK52E cells. RGN may have a physiological role in the regulation of the proliferation of rat kidney proximal tubular epithelial cells.

The effect of overexpression of RGN on cell proliferation is suppressed after culture with butyrate, roscovitine and sulforaphane, which induce cell cycle arrest. Butyrate induces an inhibition of G1 progression. Roscovitine is a potent and selective inhibitor of the cyclin-dependent kinase cdc2, cdk2 and cdk5 and can arrest in G1 and accumulate in G2 phases of cell cycle. Sulforaphane can induce G2/M phase cell cycle arrest. The effect of butyrate, rosvatin or sulforaphan, which inhibits the proliferation of wild-type NRK52E cells, is not seen in the transfectants overexpressing RGN. This finding suggests that endogenous RGN induces G1 and G2/M phase cell cycle arrest.

The expression of RGN in proximal tubular epithelial cells is also suppressed after culture with PD98059, staurosporine or dibu- caine, which is an inhibitor of various protein kinases. These inhibitions are not seen in the transfectants that are overexpressing endogenous RGN. The suppressive effect of endogenous RGN on cell proliferation may result from the inhibitory effect of RGN on various protein kinases that are involved in the stimulation of cell proliferation. In addition, culture...
with wortmannin, an inhibitor of PI3-kinase, represses proliferation of NRK52E cells. This repression is not observed in the transfectants. Endogenous RGN may inhibit PI3-kinase and partly contribute to suppression of cell proliferation in NRK52E cells. The proliferation of wild-type NRK52E cells is repressed in the presence of Bay K 8644, an agonist of calcium entry into cells. This effect is not seen in the transfectants. This may support the view that endogenous RGN suppresses apoptotic cell death, which is mediated through an increase in intracellular Ca\(^{2+}\) levels due to maintaining intracellular calcium homeostasis.

Moreover, the gene expression for proteins that are involved in cell proliferation and cell cycle has been shown to regulate in the transfectants. The expression of c-jun and checkpoint kinase 2 (chk2) mRNAs has been found to be suppressed in the transfectants. The expression of p53 mRNA is enhanced in the transfectants, while the expression of c-myc, c-fos, cdc2 and p21mRNAs is not changed in the transfectants. The decrease in c-jun and chk2 mRNA expressions may partly contribute to suppression of cell proliferation, which is induced in NRK52E cells overexpressing RGN. The expression of the tumour suppressor gene p53 mRNA in the transfectants may play a partial role in the retardation of proliferation of NRK52E cells. RGN has been shown to localize in the nucleus of NRK52E cells. RGN may repress the transcription of multiple genes that are related to cell proliferation in NRK52E cells.

Thus, overexpression of endogenous RGN has been demonstrated to reveal a suppressive effect on cell proliferation due to inducing G1 and G2/M phase cell cycle arrest in NRK52E cells; the effect of RGN may be mediated through a decrease in various Ca\(^{2+}\) signalling-dependent protein kinases and PI3-kinase activities and suppression of c-jun and chk2 mRNAs expression or the enhancement of p53 mRNA expression.

**Overexpression of endogenous RGN suppresses apoptotic cell death**

The role of endogenous RGN in apoptotic cell death is shown in NRK52E cells overexpressing RGN. The number of wild-type cells was decreased after culture for 42–72 hours in the presence of TNF-α (0.1 or 1.0 ng/ml of medium), lipopolysaccharide (LPS; 0.1 or 1.0 μg/ml), Bay K 8644 (1–100 nM), or thapsigargin (1–100 nM). These effects are not seen in the transfectants that overexpress RGN. DNA fragmentation was induced after culture with LPS, Bay K 8644 or thapsigargin, and these effects are depressed in the transfectants. Overexpression of RGN has been found to have a suppressive effect on apoptotic cell death induced by TNF-α, LPS, Bay K 8644 or thapsigargin in NRK52E cells.

TNF-α, LPS or Bay K 8644-induced cell death in NRK52E cells is repressed in the presence of caspase-3 inhibitor. LPS or Bay K 8644-induced cell death is blocked by Nω-nitro-L-arginine methyl ester (NAME), an inhibitor of NO synthase, in NRK52E cells. Thapsigargin-induced cell death is repressed in the presence of caspase-3 inhibitor or NAME. NO may be important as a signalling factor in many cell types and plays a role in apoptosis of hepatoma cells. NO is produced by NO synthase that is activated by Ca\(^{2+}\)/calmodulin. Bay K 8644-induced calcium entry into the cells induces cell death. The effect of RGN in suppressing apoptotic cell death may be mediated through its action on many intracellular signalling pathways in NRK52E cells. Bcl-2 is a suppressor in apoptotic cell death. Apaf-1 participates in activation of caspase-3. Akt-1 is involved in survival signalling pathways for cell death. Overexpression of RGN has been found to produce a remarkable elevation of Bcl-2 mRNA expression in NRK52E cells and slightly stimulated Akt-1 mRNA expression in the cells. Apaf-1, caspase-3 or G3PDH mRNA expressions are not altered in the transfectants. The enhancement of Bcl-2 mRNA expression may contribute to the suppression of apoptotic cell death in NRK52E cells overexpressing RGN. Endogenous RGN may play a role in the regulation of Bcl-2 gene expression in NRK52E cells. The expression of caspase-3 mRNA in NRK52E cells is enhanced after culture with TNF-α. This enhancement is suppressed in the transfectants. The mechanism by which RGN suppresses TNF-α-induced cell death may be partly related to the decrease in caspase-3 mRNA expression in the transfectants.

Culture with LPS caused a decrease in Bcl-2 mRNA expression in NRK52E cells, suggesting that this decrease leads to LPS-induced cell death. Overexpression of endogenous RGN enhances Bcl-2 mRNA expression, and this effect is also seen in the presence of LPS. LPS-stimulated Apaf-1 mRNA expression is suppressed in the transfectants. This may lead to suppression of LPS-induced cell death in NRK52E cells. Culture with Bay K 8644 or thapsigargin has been found to produce an increase in caspase-3 mRNA expression in wild-type NRK52E cells, suggesting that the increase in the gene expression induces apoptotic cell death. This increase is depressed in the transfectants. RGN may have a suppressive effect on caspase-3 mRNA expression enhanced by Bay K 8644 or thapsigargin in NRK52E cells. Thus, overexpression of endogenous RGN has been found to enhance the expression of Bcl-2 and Akt-1 mRNAs and to suppress the expression of caspase-3 and Apaf-1 mRNAs in NRK52E cells.

Many toxic factors have been reported to induce renal failure due to stimulating apoptotic cell death. Overexpression of RGN has a suppressive effect on apoptotic cell death induced by various factors (including TNF-α, LPS, Bay K 8644 or thapsigargin) in NRK52E cells. Endogenous
RGN may play an important role as a suppressor in the development of apoptotic cell death in the kidney proximal tubular epithelial cells.

**Overexpression of endogenous RGN regulates gene expression of mineral ion transport-related proteins**

Overexpression of endogenous RGN has been shown to increase in rat outer medullary K⁺ channel (ROMK) mRNA expression in NRK52E cells, while it did not have an effect on Na⁺, K⁺-ATPase and epithelial sodium channel (ENaC) mRNA expressions. Culture with aldosterone causes an increase in ENaC, Na⁺, K⁺-ATPase and ROMK mRNA expression in wild-type NRK52E cells. The expression of these genes has been reported to increase after treatment with aldosterone in rat kidney cells. The effect of aldosterone in increasing ENaC and Na⁺, K⁺-ATPase mRNA expressions is not observed in the transfectants overexpressing RGN. However, overexpression of endogenous RGN reveals a stimulatory effect on ROMK mRNA expression in the transfectants untreated with aldosterone. Culture with aldosterone does not enhance ROMK mRNA expression in the transfectants cultured with serum withdrawal. Aldosterone has been shown to up-regulate RGN mRNA expression in NRK52E cells. The stimulatory effect of aldosterone on ENaC and Na⁺, K⁺-ATPase mRNA expressions may be partly mediated through endogenous RGN in NRK52E cells. The expression of type II Na-Pi cotransporter (NaPi-IIa) and angiotensinogen mRNAs is not changed in NRK52E cells over-expressing RGN, suggesting that endogenous RGN does not have effects on NaPi-IIa and angiotensinogen mRNA expressions in NRK52E cells.

Overexpression of endogenous RGN has also been found to have suppressive effects on the gene expression of L-type Ca²⁺ channel and calcium-sensing receptor (CaR), which regulate intracellular Ca²⁺ signalling in NRK52E cells. The blockade of Ca²⁺ influx through L-type Ca²⁺ channels has been shown to attenuate mitochondrial injury and apoptosis in hypoxia of renal tubular cells. The entry of Ca²⁺ through L-type Ca²⁺ channels induces mitochondrial disruption and cell death. CaR participates in the regulation of renal Ca²⁺ transport. RGN may regulate the intracellular Ca²⁺-signalling pathway, which is mediated through its suppressive effect on L-type Ca²⁺ channel or CaR mRNA expressions in the kidney proximal tubular epithelial cells.

The expression of RGN mRNA in NRK52E cells has been shown to enhance after PTH treatment, suggesting that RGN partly mediates cellular response for PTH in kidney cells. Overexpression of endogenous RGN did not attenuate the expression of L-type Ca²⁺ channel or CaR mRNAs, which is decreased after PTH treatment, in NRK52E cells. Endogenous RGN is found to suppress L-type Ca²⁺ channel or CaR mRNA expressions in NRK52E cells. RGN may play a role as a mediator in the cellular response after stimulation with PTH in NRK52E cells.

RGN has been shown to play a role in the regulation of intracellular Ca²⁺ transport; the protein activates Ca²⁺-pumping enzymes (Ca²⁺-ATPase) in the basolateral membranes, mitochondria and microsomes in the rat kidney cortex. RGN may regulate intracellular Ca²⁺ homeostasis in the kidney proximal tubular epithelial cells that Ca²⁺ is passed through transcellular transport. Moreover, RGN has been shown to suppress the expression of L-type Ca²⁺ channel or CaR mRNAs in NRK52E cells. Interestingly, calcium has been demonstrated to be present through numerous steps of tubulogenesis and nephron induction during embryonic development of the kidney. Several calcium-binding proteins such as RGN and calbindin-D28k are commonly used to label pronephric tubules and metanephric ureteral epithelium.

RGN may play a physiological role in the regulation of intracellular Ca²⁺ homeostasis, mineral transport, cell proliferation and apoptotic cell death in the kidney proximal tubular epithelial cells. RGN, which is translocated into the nucleus through the Ca²⁺ signalling pathway, regulates the gene expression of their many related proteins. The role of RGN in the regulation of the kidney proximal tubular epithelial cells is summarized in Figure 2.

**Overexpression of endogenous RGN suppresses TNF-α and TGF-β1-mediated cell responses**

Overexpression of endogenous RGN, moreover, has been found to have a suppressive effect on cell responses that are mediated through the signalling process following stimulation with TNF-α or TGF-β1 in NRK52E cells. RGN mRNA expression is suppressed after TNF-α or TGF-β1 in NRK52E cells. However, overexpression of endogenous RGN suppressed apoptotic cell death induced by TNF-α or TGF-β1 that is mediated through caspase-3 in NRK52E cells. RGN localizes in the nucleus of NRK52E cells and may inhibit nuclear DNA fragmentation that is partly related to caspase-3.

The effect of Ca²⁺/calmodulin in increasing NO synthase activity in NRK52E cells is depressed in the transfectants overexpressing RGN. NO synthase activity is increased in wild-type NRK52E cells after culture with TNF-α, although culture with TGF-β1 does not have an effect on the enzyme activity. Overexpression of endogenous RGN has a suppressive effect on the increase in NO synthase activity in NRK52E cells cultured with TNF-α. The suppressive effect of RGN on TNF-α-induced cell death may be partly involved in its inhibitory effect on NO synthase activity in NRK52E cells.

Cultures with TNF-α and TGF-β1 causes a remarkable increase in α-smooth muscle actin level in NRK52E cells.
Interestingly, this increase is not seen in the transfectants. In addition, the expression of α-smooth muscle actin is markedly suppressed in the transfectants cultured without TNF-α or TGF-β1. These findings suggest that overexpression of endogenous RGN suppresses many enzyme activations, which are mediated through Ca2+ and other signalling pathways. Nuclear localisation of RGN is promoted through protein kinase C-related signalling. Nuclear RGN regulates the gene expression of many proteins that mediate mineral transport, cell proliferation and apoptotic cell death.

Figure 2: RGN plays a multifunctional role in the kidney proximal tubular epithelial cells. Endogenous RGN plays a role in the regulation of mineral transport, cell proliferation and apoptotic cell death in the kidney proximal tubular epithelial cells. The expression of rgn is stimulated through Ca2+ signalling, parathyroid hormone or aldosterone, which regulates mineral transport in the cells. RGN suppresses many enzyme activations, which are mediated through Ca2+ and other signalling pathways. Nuclear localisation of RGN is promoted through protein kinase C-related signalling. Nuclear RGN regulates the gene expression of many proteins that mediate mineral transport, cell proliferation and apoptotic cell death.

The expression of RGN mRNA is suppressed in the renal cortex of rats with chemically induced kidney damage. RGN mRNA expression is markedly reduced in the kidney cortex of rats that received a single intraperitoneal administration of cisplatin and cephaloridine; these administrations induced a remarkable accumulation of proteins.

Involvement of RGN in kidney disorder

There is growing evidence that the suppression of rgn expression is involved in the development of kidney disorder. The tubular epithelial cells in rgn knockout mice aged 12 months have been shown to cause deposition of lipofuscin and presence of senescence-associated beta-galactosidase. RGN mRNA expression is suppressed in the kidney cortex of rats after saline intake for 7 days, which causes an increase in the serum calcium and BUN concentrations as an index of kidney disorder. RGN mRNA expression is suppressed in SHR, suggesting an involvement in a hypertensive state.

The expression of RGN mRNA is suppressed in the renal cortex of rats with chemically induced kidney damage. RGN mRNA expression is markedly reduced in the kidney cortex of rats that received a single intraperitoneal administration of cisplatin and cephaloridine; these administrations induced a remarkable accumulation of proteins.
of calcium in the kidney cortex and a corresponding elevation of BUN at 1–3 days after administration\textsuperscript{43}. Kidney toxic chemicals-induced suppression of RGN expression may lead to renal disease.

The expression of rgn has been shown to be down-regulated in rat kidney tissues after treatment with many nephrotoxins\textsuperscript{10-13}. The proximal tubule segment-specific nephrotoxins, namely hexachloro-1,3,5,7,8-pentabromodibenzo-p-dioxin (PBDD), specific for S(3) segment (pars recta), and potassium dichromate (chromate) specific for S(1)–S(2) segments (pars convoluta) were used in this study\textsuperscript{105}. RGN is down-regulated after HCBD administration with low dose, whereas chromate causes the same effect at high doses only\textsuperscript{105}. Glutamine synthase activity in the kidney cortex shows a similar behaviour even if sensitive to low doses of chromate also, whereas BUN and creatinine increase after a high dose of both chemicals only\textsuperscript{105}. The expression of rgn appears to be a sensitive genomic marker to evaluate the renal impairment caused by chemicals and its down-regulation seems to be related to damage, early by chemicals and its down-regulation may be involved in tubulogenesis and nephron induction. As described above, kidney RGN expression has been shown to be down-regulated with renal disorder induced by various drugs. Depression of the rgn expression in kidney cells may play a pathophysiological role in development of renal disorders.

Conclusion

Calcium, which plays a multifunctional role in many biological systems, plays a pivotal role during embryonic development of kidneys and is present through numerous steps of tubulogenesis and nephron induction from the formation of a simple kidney in amphibian larvae, the pronephron, to the formation of the more complex mammalian kidney, the metanephrons. RGN, which is a Ca\textsuperscript{2+}-binding protein, is greatly expressed in proximal tubular cells. Several Ca\textsuperscript{2+}-binding proteins such as RGN and calbindin-D28k are commonly used to label proxenphric tubules and metanephric ureteral epithelium. RGN is focused on Ca\textsuperscript{2+}-binding proteins and Ca\textsuperscript{2+} sensors that are involved in renal organogenesis in the link between Ca\textsuperscript{2+}-dependent signals and polycystins. RGN may be involved in tubulogenesis and nephron induction.

RGN plays a physiological role in the regulation of Ca\textsuperscript{2+} homeostasis due to activating membranous Ca\textsuperscript{2+}-pump enzymes in the kidney proximal tubular epithelial cells, which participate in transcellular transport to reabsorption of calcium from filtrated urinary calcium. RGN suppresses the activities of proteins: calcium homeostasis (regucalcin and calbindin), cytokine receptor (vimentin and caldesmon), response to hypoxia and mitochondrial function (prolyl 4-hydroxylase, proteasome and NADH dehydrogenase) and cell metabolism (kidney aminoacylase, pyrroate dehydrogenase and fructose-1, 6-bisphosphate)\textsuperscript{108}. RGN increased in the urine with kidney disorder and may be useful as a biomarker in kidney disorders.

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of various Ca\textsuperscript{2+}-dependent protein kinases and protein phosphatases, NO synthase and cAMP phosphodiesterase, which are related to intracellular signalling pathways. Moreover, RGN suppresses nuclear DNA synthesis in the kidney cortex and activates thiol proteases. Thus, RGN plays a pivotal role in the regulation of kidney cell function. Further findings of the role of RGN in kidney cell regulation are expected.

RGN localizes to the nucleus, and it has a regulatory effect on the gene expression of various proteins in the nucleus of kidney tubular epithelial cells. RGN regulates the gene expressions of mineral transport-related proteins, Smad 2 and NF-κB, and cell proliferation and apoptosis-related proteins. Interestingly, RGN suppresses the gene expression of TNF-α and TGF-β1-stimulated α-smooth muscle actin that induces transdifferentiation to renal fibrosis, suggesting an involvement in the development of renal disease. Future studies will determine the molecular mechanism by which RGN regulates gene expression in the nucleus of kidney tubular epithelial cells.

The expression of rgn is suppressed in various pathophysiological states with hypertensive state or drugs that induce kidney disorder. The analysis for proteome and differential gene expressions of kidney tubular epithelial cells.

The expression of rgn is suppressed in various pathophysiological states with hypertensive state or drugs that induce kidney disorder. The analysis for proteome and differential gene expressions of kidney tubular epithelial cells.

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