Role of response gene to complement 32 in diseases

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Abstract

The role of response gene to complement (RGC)-32 as a cell cycle regulator has been attributed to its ability to activate cdc2 kinases and to induce S-phase entry and mitosis. However, recent studies revealed novel functions for RGC-32 in diverse processes such as cellular differentiation, inflammation, and fibrosis. Besides responding to C5b-9 stimulation, RGC-32 expression is also induced by growth factors, hormones, and cytokines. Transforming growth factor β activates RGC-32 through Smad and RhoA signaling, thus initiating smooth muscle cell differentiation. Accumulating evidence has drawn attention to the deregulated expression of RGC-32 in human malignancies, hyper-immunoglobulin E syndrome, and fibrosis. RGC-32 expression is up-regulated in cutaneous T cell lymphoma and colon, ovarian, and breast cancer, but down-regulated in invasive prostate cancer, multiple myeloma, and drug-resistant glioblastoma. A better understanding of the mechanism by which RGC-32 contributes to the pathogenesis of these diseases will provide new insights into its therapeutic potential. In this review we provide an overview of this field and discuss the most recent research on RGC-32.

Key words: RGC-32, cell cycle, differentiation, cancer, cdc2.

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INTRODUCTION

The response gene to complement (RGC)-32 gene was first cloned from rat oligodendrocytes by differential display during a search by Badea et al. [4] for genes that were differentially expressed in response to complement activation. The human and mouse RGC-32 genes were later cloned by the same group [3]. The human RGC-32 gene is located on chromosome 13 and encodes a 137-amino-acid protein with 92% similarity to both the rat and mouse proteins [3]. RGC-32 mRNA is abundantly expressed in the placenta, skeletal muscle, kidney, pancreas, liver, and aortic endothelial cells [3]. RGC-32 is weakly expressed in the heart and brain, and is not expressed in the lungs [3]. The RGC-32 protein has been detected in human brain, heart, and liver by Western blotting [3].

Since the initial report describing RGC-32 as a gene induced by complement, several studies have reported changes in RGC-32 expression in a wide range of cell lines and tissues in response to a variety of stimuli (Table 1). RGC-32 mRNA expression is induced by complement activation and C5b-9 [3, 15], steroid hormones [10], growth factors [15], and serum (Fig. 1). Its expression is significantly higher in human embryonic

stem cells cultured in serum-containing medium than in those grown in serum-replacement medium [41]. RGC-32 seems to play a role in tumorigenesis and immunity, since it is involved in the regulation of the cell cycle and of cellular differentiation.

In this review we will summarize the recent advances in RGC-32 research and discuss its involvement in various diseases.

THE ROLE OF RGC-32 IN CELL PROLIFERATION AND REGENERATION

The progression of cells through the cell cycle is orchestrated by a number of cyclin-dependent kinases (CDKs) that are associated with specific regulatory cyclins and interact with CDK inhibitors (CKIs). CKIs function as negative regulators of the cell cycle and inhibit cyclin-CDK activity. CDK4-cyclin D1 and CDK6-cyclin D1 are involved in the progression to G1 in response to growth factor stimulation, while CDK2-cyclin E controls the G1/S transition. Cyclin A associates with CDK2 and regulates S-phase entry and DNA replication. Accumulation of CDC2-cyclin B1 and complexes during G2 enables the cell to enter mitosis [40].

Table 1. Stimuli influencing RGC-32 expression

Stimulus	RGC-32 expression	Tissue/cell line	References
Complement activation, C5b-9	Increased	Rat oligodendrocytes, human aortic smooth muscle cells	3, 4
Fetal bovine serum	Increased	Human embryonic stem cells	41
Growth factors	Increased	Human aortic endothelial cells	15
TGF- β	Increased	Monc-1 neural crest cell line	28
Corticosteroids	Increased	Human trabecular cells	35
		Human lens epithelial cells	21
		Rat skeletal muscle, kidney cells	1, 2
		Rat cardiomyocytes	10
Luteinizing hormone	Increased	Rat periovulatory follicles	22
Human chorionic gonadotropin	Increased	Rodent luteinizing granulosa cells	23
17 β -estradiol	Increased	Human endometrial tissue	12
		Rat somatotrophic cell line	17
Progesterone	Increased	Human endometrial cell line HECS-26S	8, 53
		Human endometrial tissue	12
Oxandrolone	Decreased	Human gastrocnemian muscle samples	5
Interleukin-1 β	Decreased	Pancreatic islet β cell line	34
Interferon- α 2b	Decreased	Human peripheral blood mononuclear cells	50

Sublytic C5b-9 induces cell cycle activation together with an increase in CDK4, CDK2, CDC2 [31, 37], and RGC-32 expression [3, 15]. RGC-32 has been found to act as a substrate and regulator of CDC2 activity [3]. RGC-32 directly binds to CDC2 and up-regulates its kinase activity, as demonstrated *in vitro* by glutathione S-transferase pull-down assays and *in vivo* by immunoprecipitation and Western blotting. The phosphorylation of RGC-32 at Thr-91 is critical to its ability to stimulate CDC2- kinase activity [3].

Overexpression of RGC-32 in the OLG-C6 glioma cell line leads to an increase in DNA synthesis in response to serum growth factors [4]. RGC-32 is located

in the cytoplasm and is translocated to the nucleus when smooth muscle cells are exposed to activated complement [3]. Overexpression of the protein in human aortic smooth muscle cells leads to S-phase and G2/M entry in unstimulated cells (cultured in serum- and growth factor-free medium), and exposure to C5b-9 further increases the G2/M progression. These data indicate that RGC-32 is able to induce S-phase entry and mitosis in smooth muscle cells and that C5b-9-mediated activation of the cell cycle may be dependent on the induction of RGC-32 and its effects on CDC2-kinase [3] (Fig. 2).

Additional evidence that RGC-32 plays an important part in cellular proliferation comes from gene array

Fig. 1. Induction of RGC-32 mRNA in aortic smooth muscle cells. **A** – human aortic smooth muscle cells were starved in serum- and growth factor-free medium for 18 h, then stimulated with serum C5b-9 or 10% fetal bovine serum (FBS) for 3 h. RGC-32 mRNA levels were determined by Northern blotting. As a control, normal human serum treated with K76 COONa (C5D) was used to block C5 cleavage and C5b-9 assembly. **B** – densitometric scanning was performed to determine the RGC-32 expression with respect to actin. Serum C5b-9 and FBS were able to induce a significant increase in RGC-32 mRNA expression, while a small increase was induced by C5D. The increase induced by C5D was most probably dependent on serum growth factors.

Fig. 2. Proposed role for RGC-32 in cell cycle activation by C5b-9. We propose that ERK1-activated C5b-9 induces phosphorylation of RGC-32. RGC-32 phosphorylation results in CDC2 activation. This is followed by an increase in CDC2 activity, nuclear translocation of the CDC2/Cyclin B1/RGC-32 complex, and cell cycle activation. p27 inhibits activation of CDC2/Cyclin B1/RGC-32.

studies. One such study was performed by Oram et al. [32] on testis-specific protein Y-encoded (TSPY), a repeat gene mapped to the oncogenic gonadoblastoma locus on the Y chromosome that enhances cellular proliferation *in vitro* and carcinogenesis *in vivo*. They found that ectopic expression of the TSPY gene shortens the G2/M transition and up-regulates RGC-32 mRNA expression as well as other pro-growth genes and oncogenes, while it down-regulates cell cycle inhibitors [32]. The results of another gene array study has also indicated that a gene homologous to RGC-32 (Olf14k04) is highly expressed during the early stage of fin regeneration and blastema formation in the medaka (*Oryzias latipes*), a teleost fish [26]. In addition, RGC-32 mRNA expression has been shown to increase during pancreatic tissue regeneration after pancreatectomy [29]. Increased levels of RGC-32 mRNA have been found during the structural and functional remodeling that takes place in the cardiac tissue when a left ventricular assist device support is implanted in congestive heart failure patients [6]. As part of this gene signature, RGC-32 was found to be up-regulated together with several genes that are related to the phosphatidylinositol 3-kinase/forkhead protein-signaling pathway and are important for cell cycle activation [6]. In addition, RGC-32 was also found to be up-regulated as part of the gene expression signature in exercise-induced cardiac hypertrophy in rats [44]. Taken together, these data suggest that RGC-32 plays an important role in regeneration and remodeling.

It is noteworthy that RGC-32 expression has been found to be down-regulated in fibroblasts obtained from older human individuals as compared with younger ones

[27], suggesting that the RGC-32 level decreases during senescence. We have shown that SP600125 (a JNK MAPK inhibitor) and PD98059 (an MEK1 inhibitor) inhibit RGC-32 mRNA expression in human aortic endothelial cells (Fig. 3). The involvement of the MAPK pathway in RGC-32 mRNA regulation is also supported by the results of Viemann et al. [51], who showed that the p38 MAPK inhibitor SB202190 blocks the basal expression of the RGC-32 gene in human umbilical vein-derived endothelial cells. These data indicate that both JNK and ERK1 are required for the expression of RGC-32 mRNA. Thus RGC-32 may enhance cell proliferation by activating CDKs such as CDC2 and by inducing S-phase entry and mitosis.

RGC-32 AND CELLULAR DIFFERENTIATION

Given the involvement of RGC-32 in cellular proliferation, it is reasonable to hypothesize that this protein also has an influence on the differentiation process. In an attempt to identify downstream targets of transforming growth factor (TGF)- β -induced smooth muscle cell differentiation, Li et al. [28] performed a microarray analysis of TGF- β -treated neural crest cells and found that RGC-32 expression was markedly increased (50-fold) after 24 h of treatment. Furthermore, RGC-32 knockdown with siRNA resulted in the inhibition of three smooth muscle cell marker genes: α -SMA, SM22 α , and calponin. In contrast, overexpression of RGC-32 in neural crest cells and pluripotent C3H10T1/2 stem cells enhanced the TGF- β -induced promoter activities of α -SMA, SM22 α , and SM-MHC. Since both Smad and RhoA signaling are necessary for TGF- β -induced smooth muscle cell differentiation from neural crest cells, the authors proposed the following model for the role of RGC-32 in smooth muscle cell differentiation: TGF- β activates Smad and RhoA signaling, which stimulates RGC-32; RGC-32 switches on smooth muscle gene transcription and thereby initiates the differentiation of these cells [28].

Another clue regarding the potential involvement of RGC-32 in differentiation has been provided by Takeda's study on the effects of NUP98-HOXA9 on human CD34⁺ hematopoietic cells [47]. NUP98-HOXA9 is a chimeric protein that results from a t(7;11)(p15;p15) chromosomal translocation and is thought to be involved in the pathogenesis of leukemia. When expressed in human CD34⁺ cells by retroviral transduction, it has a biphasic effect: it produces an initial phase of early growth suppression, followed by induction of long-term proliferation and, most importantly, impaired differentiation. In this late phase, Takeda et al. [47] noted the repression of several putative tumor suppressors and observed that the expression of genes involved in hematopoietic differentiation, including RGC-32, was equally suppressed.

RGC-32 mRNA expression has also been found to be up-regulated in the late stages of the differentiation

Fig. 3. Induction of RGC-32 mRNA expression by C5b-9 is dependent upon JNK and ERK activation. **A** – smooth muscle cells were starved in serum- and growth factor-free medium for 18 h and then pretreated with SP600125 (SP) to inhibit JNK1 or PD98059 (PD) to inhibit MEK1 for 45 min. The cells were then stimulated with anti-HLA I antibody plus normal human serum (serum C5b-9) for 3 h and total RNA was extracted. RGC-32 mRNA expression was determined by Northern blotting. **B** – the results in panel **A** are shown as density ratios to actin.

of both bone marrow and adipose tissue-derived human mesenchymal stem cells. The highest up-regulation (26.86 fold) was seen during osteogenesis involving bone marrow stem cells, suggesting that RGC-32 is actively involved in osteogenic differentiation [30].

Taken together, these studies provide evidence that RGC-32 plays a role in cellular differentiation, including the initiation of smooth muscle cell differentiation.

RGC-32 AND CANCER

Given that RGC-32 plays a role in promoting cellular proliferation and that perturbations in the cell cycle are commonly described in malignant transformation, it is not surprising that RGC-32 expression has been detected in a wide range of human cancers. Although most cancer cell lines lack RGC-32, most tumor tissue samples express this protein [16, 39]. RGC-32 expression has been found to be up-regulated in colon [16], ovarian [13], breast [16, 25], and prostate [16] cancers and cutaneous T cell lymphoma [19] (Table 2). In patients with colon cancer, higher RGC-32 levels have been found in tumor tissue than in normal adjacent tissue [16]. However, it has also been reported that RGC-32 mRNA expression is lower in advanced stages of primary astrocytomas [39]. The same group has also reported that RGC-32 transcription is directly regulated by p53 in glioblastoma/astrocytoma, osteoblastic, and colon cancer cell lines. Overexpression of RGC-32 in tumor cell lines was found to result in delayed G2/M progression when compared with that in control cells. RGC-32 was localized to the cytoplasm of tumor cells during interphase and concentrated in the centrosomes and spindle poles during prometaphase and metaphase. Furthermore, RGC-32 was seen to form a complex with polo-like kinase 1 (Plk1) during mitosis and was phosphorylated by Plk1, suggesting that it might negatively regulate the cell cycle [39]. These data suggest that RGC-32 can form complexes not only with CDC2, but also with Plk1 and that

the effect on the cell cycle is dependent on the kinase to which it binds in the cell.

Our immunohistochemical studies of a large number of tumor tissues [16] have indicated that RGC-32 is expressed in prostate, bladder, breast, lung, and digestive tract tumors [16]. RGC-32 protein was present in 70% of the colon carcinoma samples tested. Two major patterns of immunoreactivity were noted in colon tumors: in some tumors, reactivity was limited only to the malignant epithelial cells, whereas in others, both malignant epithelial cells and interstitial cells were positive for RGC-32 staining. In most of the neoplastic colon epithelium, RGC-32 was co-localized with the cell cycle activation marker Ki-67. Interestingly, the RGC-32 protein was absent from normal colon epithelial cells that were adjacent to the tumor [16]. This increased RGC-32 protein expression in tumors compared with normal tissue argues against its proposed tumor-suppressing role [39, 46]. It is possible, however, that RGC-32 can play a role in cell proliferation and also work as a tumor suppressor gene in certain types of cancers. RGC-32 was found to be subjected to epigenetic silencing in the Ishikawa endometrial cancer cell line [46]. RGC-32 mRNA expression was induced after 5-Aza-CdR (demethylating agent) and SAHA (histone deacetylase inhibitor) treatment. CpG islands were found within the RGC-32 promoter region, but methylation of the promoter was not detected. Conflicting data have been presented concerning the effect of p53 on RGC-32 expression. While Saigusa et al. [39] have reported an increased expression of RGC-32 after treatment with adriamycin in p53^{+/+} (HCT116 cells) but not in p53^{-/-} HCT116 cells, others have found the RGC-32 gene to be overexpressed (as are cyclin E1, cyclin E2, and cyclin G2) in irinotecan-treated p53-mutant SW620 colon cancer cells [43]. In addition, RGC-32 is one of the genes that have been found to be up-regulated after K-ras transformation of normal enterocytes [38]. *In vivo*, K-ras transformation occurs late in colon carcinogenesis and enables the transition from small to large adeno-

Table 2. RGC-32 expression in human cancers and tumor cell lines, based on gene array studies

Type of cancer	RGC-32 expression	Tissue/cell line	Real time-PCR	References
Endometrial cancer	Decreased	Ishikawa cell line	Yes	46
Ovarian cancer	Increased	Human cancer tissue	No	13
Gliomas	Decreased or deleted	Cell lines and tumor samples	Yes	39 7
Liver metastasis	Decreased	Human cancer tissue	No	20
Multiple myeloma	Decreased	Bone marrow plasma cells	No	54
Metastatic breast cancer	Increased	MDA-MB-231 cell lines	No	25
Prostate cancer	Decreased	Human cancer samples	No	33
Metastatic prostate cancer	Decreased	Human cancer samples	No	9
v-SRC down-regulated genes	Decreased	3Y1 rat fibroblasts	No	45
Ras transformed enterocytes	Increased	IEC-18 ras transformed cell line	No	38
TPSY transfected HeLa	Increased	HeLa	Yes	32
Colon cancer treated with topoisomerase I inhibitor SN-38	Increased	SW-620 (p53 defective) cells	Yes	43
Breast carcinoma	Increased	MCF-7 breast carcinoma cells	No	11

mas. Other studies using gene microarrays have shown changes in the expression of RGC-32 mRNA in tumors (Table 2). These studies have indicated that RGC-32 mRNA expression was down-regulated in some tumors, such as high-grade prostate intraepithelial neoplasia, invasive prostate cancer [33], multiple myeloma [54], and drug-resistant glioblastoma [7], but up-regulated in others, including ovarian [13] and breast [11] cancer and cutaneous T cell lymphoma [19].

Similarly, studies of RGC-32 mRNA expression in various metastatic cancers have yielded different results: RGC-32 was shown to be up-regulated in breast cancer with osteolytic metastasis [25], but down-regulated in androgen-resistant metastatic prostate cancer [9] and metastatic liver carcinoma [20]. In addition, RGC-32 was found to be preferentially expressed in micrometastases (lesions smaller than 1 mm) compared with the macrometastases of small-cell lung cancer [24].

All these data are very interesting and suggest a complex role for RGC-32 in cancer. More studies (including epigenetic work) are needed to elucidate the exact role of RGC-32 in a wide range of cancers.

RGC-32 AND HORMONES

Although RGC-32 is primarily known as a gene that is induced by complement and growth factors, recent studies have shown that hormones can also affect the expression of RGC-32 mRNA.

Response to corticosteroids

A number of studies looking at various effects of corticosteroids have reported up-regulation of RGC-32.

Treatment with corticosteroids has been shown to cause an up-regulation of prosurvival members of the bcl-xL family and RGC-32 [10]. Recent work from Almon et al. [1, 2] has singled out the RGC-32 gene as a potential “corticoid-enhanced gene,” since they observed up-regulation of RGC-32 mRNA in response to methylprednisolone in the skeletal muscle and kidney cells of Wistar rats.

Dexamethasone exposure has been found to up-regulate RGC-32 mRNA expression in human trabecular meshwork cells [35] and lens epithelial cells [21]. In contrast, oxandrolone, an anabolic steroid that reacts mainly with the androgen receptor, was found to down-regulate the expression of RGC-32 in gastrocnemius muscle samples from Duchenne muscular dystrophy patients [5].

Response to oestradiol/progesterone

RGC-32 mRNA expression has been reported to be elevated in rodent luteinizing granulosa cells following human chorionic gonadotropin stimulation [22, 23]. It has also been identified as one of the potential downstream targets of luteinizing hormone-induced RUNX1, since siRNA-mediated silencing of RUNX1 mRNA significantly reduced the level of RGC-32 [22]. The levels of RGC-32 have been found to be increased in the ovulating follicles of rodent ovaries, and it has been suggested that RGC-32 may play a role in changes in the cell cycle of luteinizing granulosa cells [22].

The identification of RGC-32 mRNA as one of the new transcripts that is selectively regulated by progesterone via progesterone receptor-A has been established by a microarray study on the human endometrial cell line HECS-26S, with subsequent confirmation by real-time PCR [53].

RGC-32 expression has also been found to be down-regulated during the early endometrial secretory phase in endometriosis in a setting of increased cellular survival and persistent expression of genes involved in DNA synthesis and mitosis; this finding is consistent with the attenuated progesterone response in endometriosis [8]. RGC-32 is also induced by estradiol, as demonstrated by gene microarrays carried out on human late proliferative phase endometrial tissue [12] and on somatolactotrophic cells of the rat pituitary gland that have been treated with 17 β -estradiol [17].

RGC-32 AND IMMUNITY

While the expression and function of RGC-32 have not been studied in a range of immune cells, a compelling piece of evidence that RGC-32 plays a role in the immune response has been provided by the gene expression studies of Tanaka et al. [49] in hyper-immunoglobulin E syndrome (HIES) patients. Using microarray analysis, they identified 33 genes that were up-regulated in unstimulated CD14⁺ cells of HIES patients compared with those of healthy patients. Among these genes, RGC-32 was the most highly expressed, suggesting that analysis of RGC-32 expression levels would be helpful in diagnosing HIES, even if the reason for this relationship between the up-regulated expression of RGC-32 and the pathogenesis of HIES is still unclear [49].

Several cytokines have been shown to modulate RGC-32 expression. These include interleukin (IL)-1 β , which is one of the cytokines involved in the destructive inflammatory process that occurs in the pancreatic islet β -cells in type I diabetes mellitus. Stimulation of the pancreatic rat insulinoma cell line (RINm5F) with high doses of IL-1 β was found to induce a down-regulation of RGC-32 mRNA, while the expression of complement component C9, C3, and complement C4b-binding protein genes was increased at 24 h [34]. Similarly, RGC-32 has been identified as one of the genes that are down-regulated in interferon- α 2b-treated peripheral blood mononuclear cells [50].

RGC-32 AND FIBROSIS

Studies investigating the development of excess fibrous connective tissue and the repair process in various organs have reported a consistent pattern of RGC-32 up-regulation [14, 18]. The first large-scale gene expression analysis after glaucoma filtration surgery in Sprague Dawley rats has confirmed the dynamics of wound healing and an increase in various mediators of scarring (an increase in the expression levels of TGF- β , connective tissue growth factor, and matrix metalloproteinases) and also indicated that RGC-32 expression levels begin to rise on day 0, reach a peak on day 5, and are still elevated on day 12. This pattern of expression for RGC-32 resembled those of TGF- β and connective

tissue growth factor, which also peaked on day 5 and were still above baseline on day 12 [14].

An earlier report that RGC-32 expression is increased in early-stage non-alcoholic hepatitis [36] has been confirmed in a more recent study indicating that RGC-32 is up-regulated in rat hepatic stellate cells; this gene analysis was designed to investigate how halofuginone alters the expression pattern of TGF- β -regulated genes that are usually overexpressed during the progression of liver fibrosis. Halofuginone is a powerful liver fibrosis inhibitor, acting upon Smad3 phosphorylation downstream of the TGF- β signaling pathway [18].

Overall, these data draw attention to the possibility that RGC-32 might play an active part in the processes of scar development and wound healing.

RGC-32 AND OTHER DISEASES

In a case report regarding autism and language deficits caused by auditory processing defects, Smith et al. [42] reported the discovery of a 9-Mb region that was deleted in the 13q12 $\rightarrow$ q13 region of chromosome 13. RGC-32 is one of the 27 genes that mapped within the deleted region, suggesting that it may function in brain development, and particularly in auditory processing.

RGC-32 mRNA was also found to be up-regulated in the cells that stably express the severe acute respiratory syndrome coronavirus (SARS-CoV) 3s protein (the major structural protein in SARS-CoV). A significant increase in the intracellular levels of fibrinogen was also noted in this 3a-expressing stable A549 cell line [48]. Also, in a microarray analysis of changes in the gene expression signature related to cold preservation/reperfusion injury in small intestinal allografts in rats, Wang et al. [52] found that RGC-32 mRNA expression was up-regulated as part of the group of "early response genes".

CONCLUSIONS

Recent data have helped us to characterize the expression and involvement of RGC-32 in cellular growth, differentiation, and tumorigenesis. RGC-32 mRNA expression is induced by glucocorticoids and sex hormones. It is also possible that RGC-32 plays an active part in immunodeficiency-related phenomena such as the hyper-immunoglobulin E syndrome. There are indications that RGC-32 activity also influences the process of wound healing and scar tissue formation. Further investigations are necessary to expand our understanding of the functions of RGC-32 and to define its therapeutic potential.

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