

Glucose deprivation regulates the progranulin–sortilin axis in PC12 cells

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Progranulin (PGRN) is a growth factor implicated in several neurodegenerative diseases, such as frontotemporal lobar degeneration. Despite its important role in the central nervous system (CNS), the mechanisms controlling PGRN expression in the CNS are largely unknown. Recent evidence, however, suggested that several stressors, such as hypoxia, acidosis, or oxidative stress, induce PGRN expression. The present study was mainly aimed at determining whether and, if so, how glucose deprivation affects PGRN expression in PC12 cells. Initially, it was found that glucose deprivation gradually induced *PGRN* gene expression in PC12 cells. To elucidate the underlying molecular mechanisms, several intracellular signalings that were modified in response to glucose deprivation were examined. Both adenosine monophosphate kinase (AMPK) activation and changes in osmotic pressure, which are modified by extracellular glucose concentration, had no effect on *PGRN* gene expression; on the other hand, p38 activation in response to glucose deprivation played an important role in inducing *PGRN* gene expression. It was also found that expression of sortilin, a PGRN receptor implicated in PGRN endocytosis, was dramatically reduced by glucose deprivation. In contrast to glucose-dependent regulation of *PGRN* gene expression, AMPK activation played a central role in reducing sortilin expression. Overall, the present study suggests that the PGRN–sortilin axis is modulated by glucose deprivation via two distinct mechanisms. As PGRN is neuroprotective, this system may represent a new neuroprotective mechanism activated by glucose deprivation in the CNS.

Progranulin (PGRN), also known as granulin epithelin precursor, PC-cell-derived growth factor (PCDGF), proepithelin or acrogranin, is a growth factor that

consists of 593 amino acids, expressed in various tissues including brain. It has been demonstrated that decreased PGRN levels in the central nervous system

Abbreviations

ACC, acetyl-CoA carboxylase; AICAR, 5-amino-imidazole-4-carboxamide-1-β-D-ribofuranoside; ALS, amyotrophic lateral sclerosis; AMPK, adenosine monophosphate kinase; ATF, activating transcription factor; CNS, central nervous system; CREB, cyclic AMP-responsive element-binding protein; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; FTLD, frontotemporal lobar degeneration; GEP, granulin epithelin precursor; GF, glucose-free; HG, high glucose; HSP27, heat-shock protein 27; LDH, lactose dehydrogenase; LG, low glucose; N2A, neuro-2 A neuroblastoma; NGF, nerve growth factor; p75NTR, p75 neurotrophin receptor; PCDGF, PC-cell-derived growth factor; PGRN, progranulin; PVDF, polyvinylidene difluoride; ROCK, Rho-associated protein kinase; SIRT1, sirtuin 1; TBS, Tris-buffered saline; VPS, vacuolar protein sorting.

(CNS) are associated with certain types of neurodegenerative disease. For example, numerous mutations in *PGRN* gene, leading to PGRN haploinsufficiency, are occasionally observed in frontotemporal lobar degeneration and amyotrophic lateral sclerosis [1–3]. Several studies clearly demonstrated that PGRN has a neuroprotective action [4–6]. Moreover, although PGRN haploinsufficiency in *Grn*^{+/-} mice displays limited neuroinflammatory phenotypes [7], evidence from *Grn*^{-/-} mice suggested that PGRN gene deficiency fosters inflammatory changes in the CNS [8,9].

Despite the importance of PGRN function in the CNS, the molecular basis of its action remains elusive. Several receptors for PGRN were recently proposed, including sortilin, which is a multifunctional protein belonging to the vacuolar protein sorting 10p domain receptor family [10]. Because sortilin endocytoses and delivers PGRN to lysosomes, sortilin seems to contribute to reducing extracellular PGRN levels [10]. In addition, several studies showed that some effects of PGRN, such as inducing neuronal outgrowth and neurotrophic properties, are independent of sortilin, suggesting that another mechanism or mechanisms may also be involved in PGRN action [11,12]. PGRN expression control is also not completely understood. Hypoxia, acidosis, and oxidative stress stimulate PGRN expression in different cell types [13–15], and PGRN can be considered as a stress-responsive factor; however, whether other forms of stress also stimulate PGRN expression is not known.

The CNS has one of the highest metabolic rates in the body. A shortage of glucose, as often observed, for example, during ischemia, decreases neuron viability and eventually causes cell death, by either necrosis or apoptosis [16–19]. Recent evidence, however, demonstrated that glucose deprivation not only leads to cell death but also exerts several protective mechanisms to counteract these adverse effects. For instance, transient glucose deprivation was reported to confer a preconditioning-like protection against subsequent detrimental stress such as ischemic reperfusion [20]. We also recently reported that glucose deprivation enhanced neuroprotective sirtuin 1 (SIRT1) expression in PC12 cells [21]. Therefore, in addition to the negative effects of glucose deprivation, these countervailing systems should also be studied to understand how change in extracellular glucose concentration determines neuron fate.

Reducing environmental glucose levels induces massive changes in intracellular components. Adenosine monophosphate kinase (AMPK) activation is one of the major intracellular responses to glucose deprivation, and can therefore be seen as an

intracellular metabolic or energy sensor [22]. AMPK is activated during glucose deprivation, mainly by increased cellular consumption of ATPs [23,24]. The activated AMPK then phosphorylates several substrates including cyclic AMP-responsive element-binding protein (CREB), thereby activating several transcription factors that facilitate cell adaptation to reduced glucose levels [25]. Glucose deprivation also enhances stress-activated mitogen-activated protein kinases (MAPKs). For example, Lauretti and Pratico showed that glucose deprivation induced p38 in neuro-2A (N2A) mouse neuroblastoma cells [26]. p38 was also activated in several oxygen/glucose deprivation models [27–29]. Similarly, the other MAPK family member, extracellular signal regulated kinase 1/2 (Erk1/2), was found to be often activated by ischemic-like insults [29–31]. Nevertheless, further research is required to understand the molecular mechanism or mechanisms underlying this stress-induced neuroprotection.

The main objective of the present study was to investigate whether and, if so, how glucose deprivation affects *PGRN* expression in PC12 cells, as a model to analyze neuronal stress-responses and neurosecretions [32–34]. In addition, we also analyzed changes in sortilin expression, which contributes to reducing extracellular PGRN levels as described above, to better understand the effects of glucose deprivation on integrated PGRN–sortilin systems.

Materials and methods

Materials

The western blot detection kit (ECL prime™ detection reagents) was purchased from GE Healthcare Inc. (Rockford, IL, USA); Dulbecco's modified Eagle's medium (DMEM), penicillin/streptomycin, and Trypsin-EDTA from Nacalai Tesque (Kyoto, Japan); cell culture equipment from Corning Inc. (Corning, NY, USA); and fetal bovine serum (FBS) from BioWest (Nuaille, France). Unless otherwise noted, all chemicals were of the purest grade available from Nacalai Tesque, Sigma Chemicals (St. Louis, MO, USA) or Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Because only an established cell line (PC12 cell) was used in this study, IRB approval was not required.

Cell culture

An established rat adrenal pheochromocytoma cell line, PC12 cell, was obtained from Dr. Shin-Ichiro Takahashi (University of Tokyo, Tokyo, Japan). The PC12 cells were

maintained in DMEM (22.5 mM glucose) containing 10% FBS, 30 $\mu\text{g}\cdot\text{mL}^{-1}$ penicillin, and 100 $\mu\text{g}\cdot\text{mL}^{-1}$ streptomycin at 37 °C under 5% CO₂ atmosphere. The medium was exchanged every 72 h. For all experiments, cells were grown on six-well plates (Corning Inc.) at a density of 5×10^4 cells per well in 3 mL growth medium, or on 96-well plates (Corning Inc.) at a density of 5×10^3 cells per well in 0.2 mL growth medium. Three days after plating, cells typically reached 50–70% confluence (Day 0). Differentiation was then induced by switching to DMEM (22.5 mM glucose) supplemented with 100 ng·mL⁻¹ NGF, 30 $\mu\text{g}\cdot\text{mL}^{-1}$ penicillin, and 100 $\mu\text{g}\cdot\text{mL}^{-1}$ streptomycin.

Western blotting

The expression and phosphorylation of each protein were analyzed by western blot analysis as previously described [35]. Briefly, cells were seeded on six-well plates at a density of 1×10^5 cells per well, differentiation was induced as described above, and the medium was switched to differentiation medium containing different amounts of glucose or sucrose (0, 5, or 22.5 mM). Cell lysates were prepared using lysis buffer [2% SDS, 1% 2-mercaptoethanol, 10% glycerol, 0.0033% bromophenol blue, and 50 mM Tris/Cl (pH 6.8)]. These cell lysates were subjected to 12% SDS-PAGE (1 : 30, bis:acrylamide). Proteins were transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore Corp., Bedford, MA, USA), and the membranes were blocked for 30 min with 3% BSA in Tris-buffered saline (TBS) containing 0.1% Tween-20. Each protein was detected with 1-h incubation with a 1 : 1000 dilution of primary antibodies: anti-phospho AMPK (Thr172), anti-AMPK, anti-phospho Acetyl-CoA carboxylase (ACC) (Ser79), anti-ACC, anti-phospho Erk1/2 (Thr202/Tyr204), anti-Erk1/2, anti-phospho p38 (Thr180/Tyr182), anti-p38, anti- β -actin antibodies (Cell Signaling Technology, Danvers, MA, USA), or anti-sortilin antibodies (Abcam plc., Cambridge, UK). Specific proteins were visualized after subsequent incubation with 1:5000 dilution of anti-mouse or rabbit IgG conjugated to horseradish peroxidase (Cell Signaling Technology) and an ECL detection procedure (GE Healthcare Inc.). Protein band intensity was quantified using IMAGEJ software (National Institutes of Health, Bethesda, MD, USA).

Quantitative PCR analysis

PC12 cells were differentiated as previously described, and then cultured in DMEM containing different concentrations of glucose (0, 5, or 22.5 mM) for 0–24 h. Total RNA was isolated using a NucleoSpin™ RNA Isolation Kit (Takara Bio Inc., Shiga, Japan) according to the manufacturer's protocol. cDNAs were synthesized from total RNA using ReverTra Ace qPCR RT Master Mix (Toyobo Co. Ltd., Osaka, Japan). Fluorescence real-time PCR analysis

was performed using a Step One instrument (Life Technologies Corporation, Grand Island, NY, USA) and an SYBR Green detection kit according to the manufacturer's protocol (KAPA Biosystems Inc., Woburn, MA, USA). PCR primers for measuring each gene comprised: rat *PGRN*, 5'-CAC TGT CCT GAT GGC TAC TCT TG-3' and 5'-CTA CCA GGA CAC TGG ACA GCA C-3'; rat *SORT1*, 5'-GAC ACA TGG AGC ATG GCA CA-3' and 5'-TGC CTC GGT CAT CAG AGG TAA AG-3'; and rat *GAPDH*, 5'-GGC ACA GTC AAG GCT GAG AAT G-3' and 5'-ATG GTG GTG AAG ACG CCA GTA-3'.

Cell death measurement

PC12 cells were seeded on 96-well plates and differentiated as previously described [36]. Percentage cell death was evaluated using the lactate dehydrogenase Cytotoxicity Detection Kit^{PLUS} (Roche Diagnostics K.K., Basel, Switzerland) according to the manufacturer's protocol.

Statistical analysis

Comparisons between treatment groups were tested using one-way ANOVA with Tukey's *post hoc* test or Student's *t*-test. Differences for which $P < 0.05$ were considered statistically significant.

Results

Glucose deprivation induced *PGRN* gene expression

To determine whether *PGRN* gene expression was modified by changes in glucose availability, differentiated PC12 cells were cultured for 24 h in DMEM containing different concentrations of glucose [high glucose (HG), 22.5 mM; low glucose (LG), 5 mM; or glucose-free (GF), 0 mM], supplemented with 100 ng·mL⁻¹ NGF. *PGRN* gene expression was then monitored by quantitative PCR as described in Materials and methods. As shown in Fig. 1A, *PGRN* gene expression was significantly increased (approximately twofold) when cells were cultured under the GF condition for 24 h ($*P < 0.05$, $n = 3$) (Fig. 1A). On the other hand, no significant differences in *PGRN* gene expression between the HG and LG groups were detected (Fig. 1A). These results strongly indicate that glucose deprivation (0 mM glucose) contributes to induction of *PGRN* gene, while the glucose-enriched condition (22.5 mM glucose) did not affect *PGRN* expression compared to the LG condition. Time course experiments revealed that at least 12–24 h of glucose deprivation is necessary for these changes to occur ($**P < 0.01$, $n = 3$) (Fig. 1B).

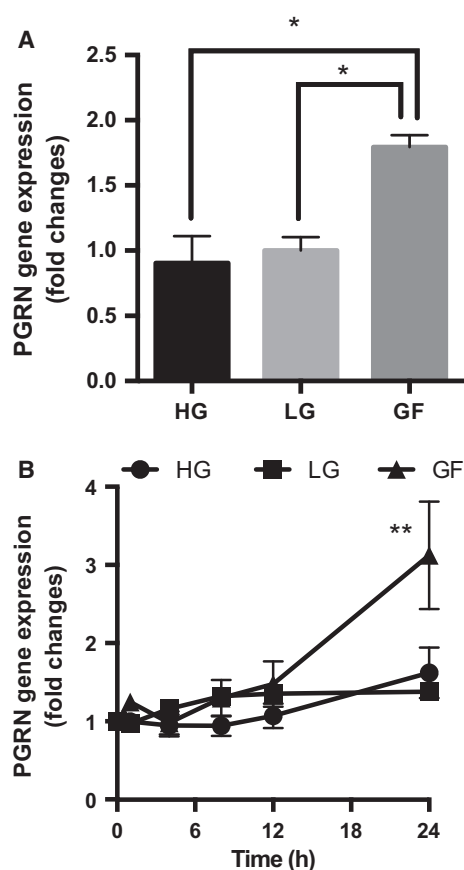


Fig. 1. Glucose deprivation induces PGRN gene expression. (A,B) PC12 cells were differentiated in differentiation medium [DMEM (22.5 mM glucose) supplemented with 100 ng·mL⁻¹ NGF] for 72 h. (A) The medium was then switched to DMEM [containing different concentrations of glucose: high glucose (HG), 22.5 mM; low glucose (LG), 5 mM; or glucose-free (GF), 0 mM] supplemented with 100 ng·mL⁻¹ NGF and incubated for 24 h. *PGRN* mRNA levels were evaluated by quantitative PCR. Corresponding graph represents fold change in *PGRN* expression normalized to *GAPDH* (LG as control). Data are mean ± SEM (**P* < 0.05, *n* = 3, one-way ANOVA). (B) The cells were cultured under HG, LG, or GF condition for the indicated times, and *PGRN* mRNA levels were measured as described above. Corresponding graph represents fold change in *PGRN* expression normalized to *GAPDH* (time 0 as control). Data are mean ± SEM (***P* < 0.01, *n* = 3, one-way ANOVA).

Change in AMPK activation or in osmotic pressure is not involved in glucose deprivation-induced PGRN gene expression

Next, we investigated the mechanisms underlying how glucose deprivation initiates *PGRN* gene expression. As described in the introduction, reduced glucose concentration is known to activate AMPK. Results also confirmed that AMPK phosphorylation, which

indicates AMPK activity, was greater under the GF condition compared then under HG (**P* < 0.05, *n* = 3) (Fig. 2A). To determine the role of AMPK in *PGRN* gene expression, we tested the effects of AICAR, a potent AMPK activator. Treatment with 0.5 mM AICAR rapidly elevated AMPK phosphorylation under HG condition (**P* < 0.05, *n* = 3, compared to 0 h) (Fig. 2B); however, 24 h of AICAR treatment did not elevate the *PGRN* gene expression (*n* = 3–5) (Fig. 2C). We also tested the effects of a specific AMPK inhibitor, compound C, on GF-induced *PGRN* gene expression; however, the expected effects of compound C were not observed (*n* = 3–5) (Fig. 2D).

As increased glucose concentration in the medium results in increased osmotic pressure, we also tested whether a high concentration of nonmetabolizable sugar (sucrose) affected *PGRN* gene expression in the same way as glucose. Differentiated PC12 cells were cultured for 24 h in DMEM without glucose but with various concentrations of sucrose [high sucrose (HS), 22.5 mM; low sucrose (LS), 5 mM; or sucrose-free (SF), 0 mM] supplemented with 100 ng·mL⁻¹ NGF, and *PGRN* gene expression was measured. No effect of sucrose on *PGRN* gene expression was observed (*n* = 4) (Fig. 2E), suggesting that change in osmotic pressure plays no significant role, but that metabolic processes and/or intracellular signalings activated by glucose influence change in *PGRN* gene expression.

p38 MAPK activation contributes to PGRN gene induction by glucose deprivation

We next studied the role of the MAPK family in *PGRN* gene expression, first checking whether the activity of each MAPK could be changed by glucose deprivation. As shown in Fig. 3A, when cells were cultured under the GF condition, p38 activity was significantly elevated [approximately threefold compared to the HG or LG conditions (***P* < 0.01, *n* = 3) (Fig. 3A)], whereas no significant change in Erk1/2 phosphorylation was observed according to glucose concentration (*n* = 3) (Fig. 3B).

To evaluate the role of p38 activation by glucose deprivation, differentiated PC12 cells were cultured for 24 h under the HG, LG, or GF conditions in the presence or absence of a p38-specific inhibitor, SB203580, and then *PGRN* gene expression was measured. As in Fig. 1A, *PGRN* gene expression was significantly elevated under the GF condition, but this effect was abolished in the presence of SB203580 (**P* < 0.05, ***P* < 0.01, *n* = 5) (Fig. 3C), suggesting that glucose

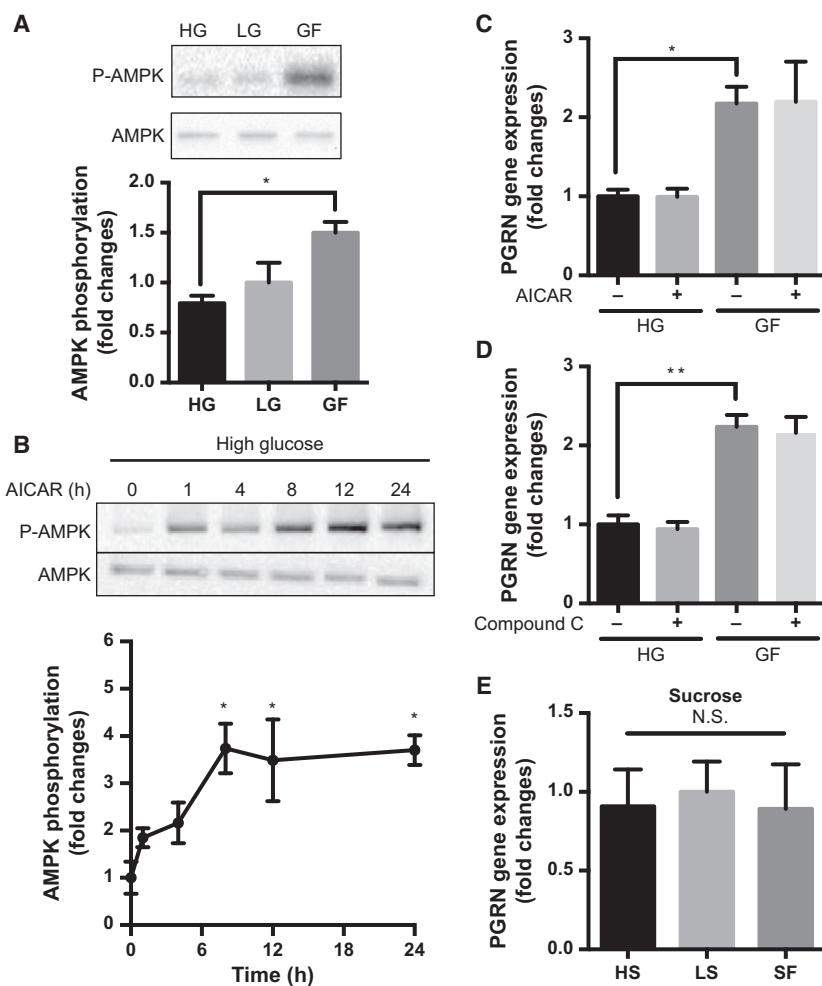


Fig. 2. Impact of AMPK activation and osmotic pressure change on PGRN gene expression induced by glucose deprivation. (A) Differentiated PC12 cells were cultured under the HG, LG, or GF conditions for 24 h, and AMPK phosphorylation was evaluated by western blot analysis. Corresponding graph represents fold change in AMPK phosphorylation normalized to total AMPK expression. Data are mean \pm SEM ($*P < 0.05$, $n = 3$, one-way ANOVA). (B) Differentiated PC12 cells were stimulated with 0.5 mM AICAR under the HG condition for the indicated times, and AMPK phosphorylation was measured as described above. Data are mean \pm SEM ($*P < 0.05$, $n = 3$, one-way ANOVA). (C) Differentiated PC12 cells were stimulated with 0.5 mM AICAR under the HG or GF condition for 24 h, and PGRN gene expression was measured by quantitative PCR (normalized to *GAPDH*). Data are mean \pm SEM ($*P < 0.05$, $n = 3$ –5, one-way ANOVA). (D) Differentiated PC12 cells were cultured under the HG or GF condition in the presence or absence of 0.25 μ M compound C for 24 h, and PGRN gene expression was measured by quantitative PCR (normalized to *GAPDH*). Data are mean \pm SEM ($**P < 0.01$, $n = 3$ –5, one-way ANOVA). (E) Differentiated PC12 cells were cultured in DMEM + 100 ng·mL⁻¹ NGF containing 25, 5, or 0 mM sucrose (HS, LS, or SF, respectively). PGRN gene expression was measured by quantitative PCR (normalized to *GAPDH*). Data are mean \pm SEM (N.S., nonsignificant; $n = 4$, one-way ANOVA).

deprivation-induced p38 activation mediates PGRN gene induction by glucose deprivation.

Glucose deprivation also alters expression of the PGRN receptor, sortilin

We also examined whether changing glucose concentration in the medium would modify the expression of

sortilin, identified as a PGRN receptor and implicated in PGRN endocytosis. Differentiated PC12 cells were again cultured for 24 h in medium containing different amounts of glucose, and the cell lysate underwent western blot analysis using anti-sortilin antibody. Sortilin protein levels were significantly decreased (approximately 0.5-fold) when cells were exposed to the GF condition compared to the HG group ($**P < 0.01$,

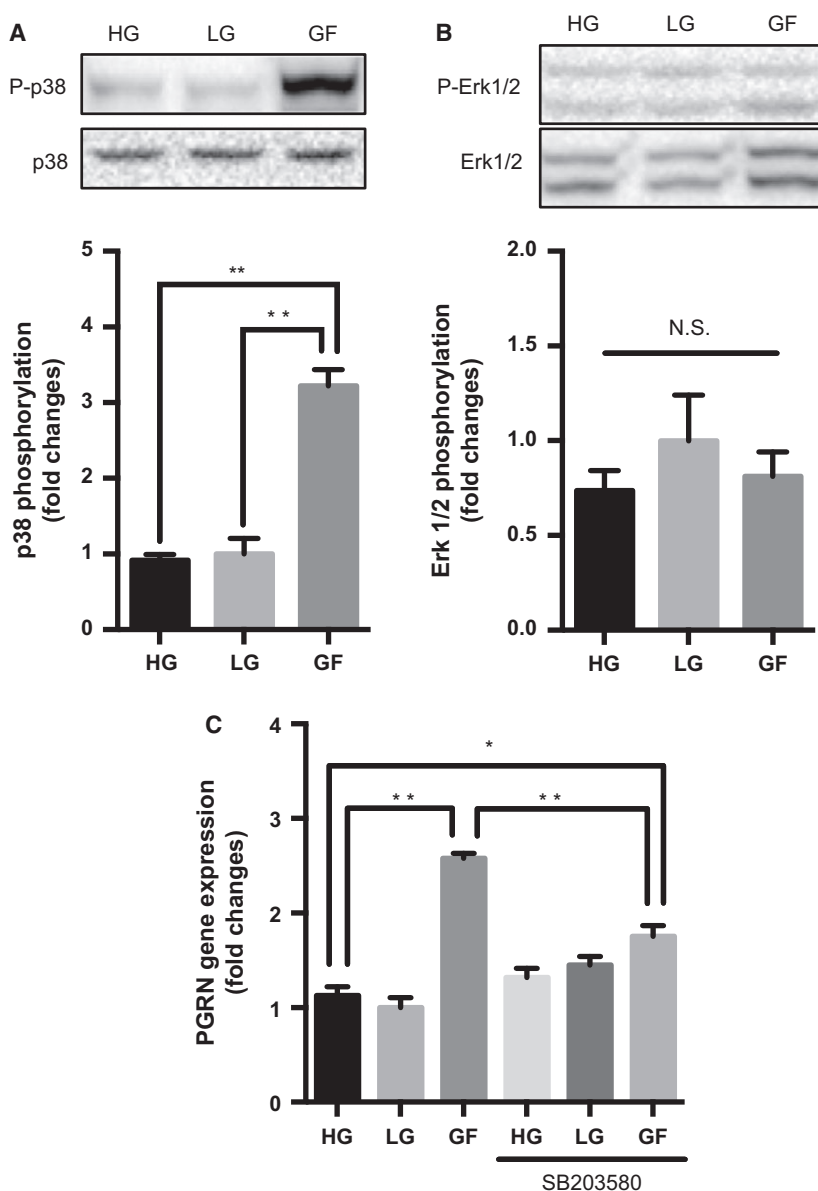


Fig. 3. Role of p38 activation in PGRN gene induction by glucose deprivation. (A, B) Differentiated PC12 cells were cultured under the HG, LG, or GF condition for 15 min, and total cell lysates were analyzed by western blot, using anti-phosphorylated p38 (P-p38) and total-p38 antibodies (A), or anti-phosphorylated Erk1/2 antibodies (B). Corresponding graphs represent fold change for each phosphorylation normalized to total expression (LG as control). Data are mean \pm SEM (** $P < 0.01$, $n = 3$, one-way ANOVA). (C) Differentiated PC12 cells were cultured under the HG, LG, or GF condition in the presence or absence of 5 μ M SB203580 for 24 h, and PGRN gene expression was measured by quantitative PCR. Corresponding graph represents fold change in PGRN expression normalized to GAPDH. Data are mean \pm SEM (* $P < 0.05$, ** $P < 0.01$, $n = 5$, one-way ANOVA).

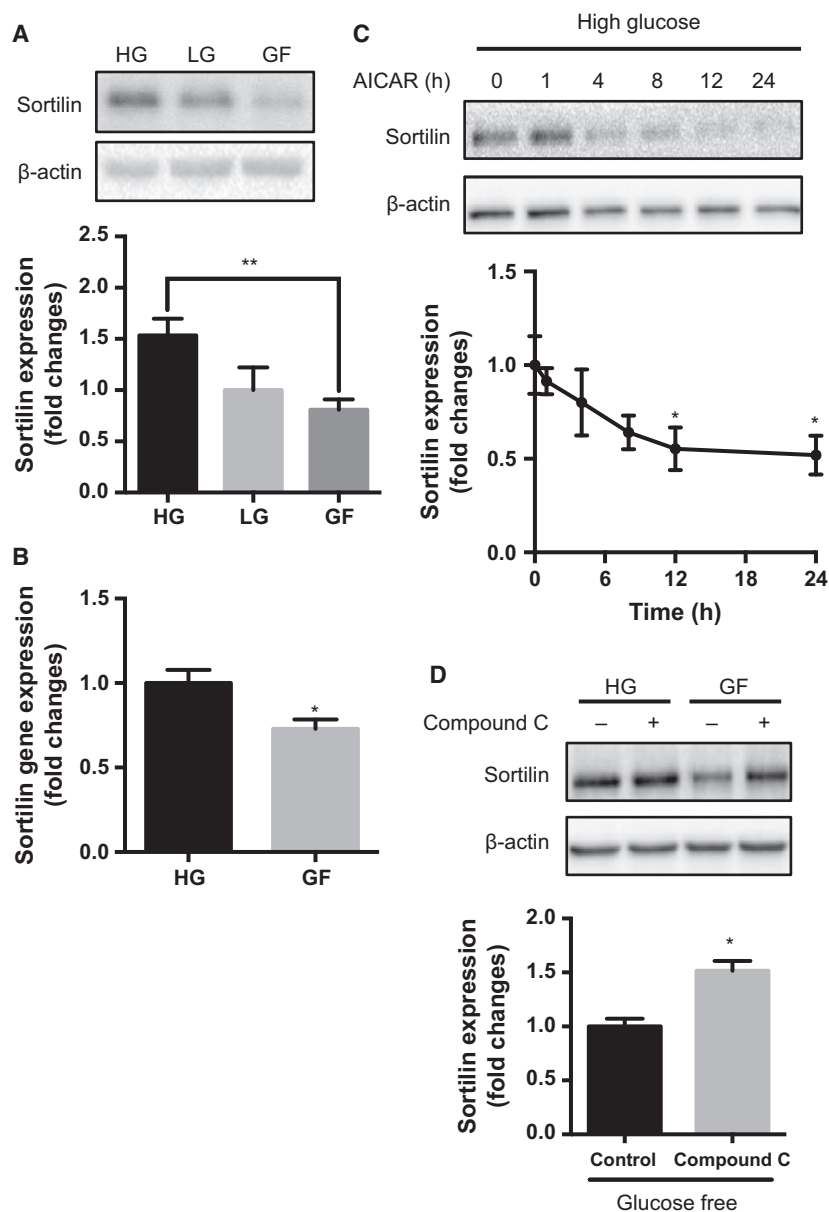
$n = 7$) (Fig. 4A). In addition, sortilin gene (*SORT1*) expression was also decreased under the GF condition, suggesting that the decrease in sortilin protein level was at least partly controlled by mRNA level (* $P < 0.05$, $n = 3$) (Fig. 4B). Interestingly, the decrease in sortilin protein level under the GF condition seemed to be regulated by AMPK, as pharmacological activation of AMPK by AICAR under the HG condition significantly decreased sortilin levels in a time-dependent manner (* $P < 0.05$, $n = 3$) (Fig. 4C). The data also confirmed that inhibition of AMPK by compound C under the GF condition increased sortilin levels (* $P < 0.05$, $n = 3$) (Fig. 4D).

Discussion

Glucose deprivation increases PGRN gene expression via p38 activation

Detrimental environments, such as glucose deprivation, are stressors for cells; at the same time, such environmental conditions activate several cell protection mechanisms. The present study demonstrated that reduction of glucose supplements induced PGRN gene expression. Moreover, expression of sortilin, implicated in PGRN endocytosis, was decreased by glucose deprivation, which

Fig. 4. Glucose deprivation decreases sortilin via AMPK activation. (A,B) Differentiated PC12 cells were cultured under the HG, LG, or GF condition for 24 h. (A) Sortilin protein levels were measured by western blot. Corresponding graph represents fold change in sortilin expression normalized to β -actin. Data are mean \pm SEM (** $P < 0.01$, $n = 7$, one-way ANOVA). (B) *SORT1* expression was measured by quantitative PCR normalized to *GAPDH*. Data are mean \pm SEM (* $P < 0.05$, $n = 3$, *t*-test). (C) Differentiated PC12 cells were stimulated with 0.5 mM AICAR under the HG condition for the indicated times, and sortilin expression was measured by western blot. Corresponding graph represents fold change in sortilin expression normalized to β -actin. Data are mean \pm SEM (* $P < 0.05$, $n = 3$, one-way ANOVA). (D) Differentiated PC12 cells were cultured under the GF condition in the presence or absence of 0.25 μ M compound C for 24 h, and sortilin expression was measured by western blot. Corresponding graph represents fold change in sortilin expression normalized to β -actin. Data are mean \pm SEM (* $P < 0.05$, $n = 3$, *t*-test).



potentially increases extracellular PGRN concentration. Overall, it could be hypothesized that glucose deprivation increased PGRN levels via two distinct mechanisms.

As described above, numerous reports have shown that PGRN has a neuroprotective property [4,6,15], and the present study confirmed that PGRN pretreatment attenuates PC12 cell death induced by high hydrogen peroxide concentrations (Fig. S1). Furthermore, recent reports strongly suggested that PGRN was a stress-responsive factor in many cell types. All of these findings support the present hypothesis that different types of stressor induce neuroprotective PGRN expression. Although further investigations are

required to know whether all stressors stimulate *PGRN* gene expression via the same mechanism or mechanisms, the present study showed that p38 activation induced by glucose deprivation plays central role in this phenomenon.

It has been reported that glucose deprivation induces p38 phosphorylation in many cell types. The present study also clearly demonstrated that glucose deprivation stimulated p38 activation in differentiated PC12 cells (Fig. 3A). On the other hand, some reports showed that p38 was activated by high concentrations of glucose, for instance in SHSY5Y human neuroblastoma cells [37]. Thus, the regulatory system of p38 activation by glucose may be dependent on cell type.

It is noteworthy that p38 is activated not only by glucose deprivation but also by various stressors, such as hypoxia, ultraviolet irradiation, heat shock, etc. [38–41], which are known to stimulate PGRN expression, as mentioned above. The present study is the first to report that stress-dependent p38 activation contributes to *PGRN* gene expression; however, intriguingly, Wang *et al.* recently reported that gastric cancer cells coming in contact with live *Helicobacter pylori* induced *PGRN* expression associated with p38 activation [42].

The next important question is to identify the downstream target or targets of p38 that underlie PGRN expression. There are several signal-transducing p38 substrates, such as MAP kinase-activated protein kinases (M2 and M3), heat shock protein 27 (HSP27), CREB, activating transcription factors, and more [43–45]. A chemical compound termed ‘crebinostat’ that robustly activates CREB-mediated transcription was shown to substantially up-regulate *PGRN* gene expression [46]. Further studies may reveal that p38-dependent CREB phosphorylation also plays a critical role in glucose deprivation-dependent *PGRN* gene induction.

As described, we identified glucose deprivation-dependent p38 activation as a major signal regulating *PGRN* gene induction (Fig. 3C); however, the significant increases in *PGRN* gene expression induced by glucose deprivation were still observed even in the presence of 5 μM SB203580, a concentration which was sufficient to inhibit p38 activity (data not shown) (Fig. 3C). These results suggested that the other signaling molecule(s) might also participate in the glucose-dependent *PGRN* expression changes.

Glucose deprivation decreases sortilin levels via AMPK activation

The present study found that expression of sortilin, one of the PGRN receptors, was significantly reduced by glucose deprivation. We recently discovered similar glucose-dependent control of sortilin expression in skeletal muscle cells [47]. Moreover, sortilin expression in liver was repressed in both genetic (*ob/ob*) mice and high-fat diet models [48], and control of sortilin expression may thus vary between tissues and organs. As described above, sortilin was reported to be a key receptor for PGRN, and induced endocytosis and lysosomal transfer of PGRN [10]. In this report, the authors expressed sortilin in COS-7 cells and applied fluorescent-labeled PGRN to examine their kinetics by using several cell biological techniques. They clearly showed there was rapid endocytosis of extracellular PGRN by cell surface sortilin to COS-7 lysosomes.

Moreover, they also showed that brain and serum PGRN levels were significantly increased in mice lacking sortilin [10]. Similarly, a genome-wide screen suggested that sortilin expression regulated PGRN levels in human plasma [49]. Overall, extracellular levels of PGRN seem to be at least partly determined by sortilin-mediated endocytosis. The present study attempted to measure PGRN levels in cellular and conditioned medium obtained from PC12 cell culture, but levels were not detectable either by ELISA or western blot analysis (data not shown). On the other hand, it is possible that PGRN concentration in the pericellular region of the cells can be changed by glucose deprivation, which is difficult to evaluate by these classical methods. We are therefore conducting further research to detect pericellular PGRN concentration by other methods.

Intriguingly, the function of sortilin is not limited to PGRN action. Sortilin makes a complex with p75 neurotrophin receptor (p75NTR), which triggers proNGF- or proBDNF-induced neuronal apoptosis [50,51]. Hence, this glucose-dependent sortilin regulation may modify not only PGRN function but also the biological function of neurotrophin precursors. Furthermore, sortilin-deficient mice showed reduced neuronal apoptosis [52], and it has also been reported that expression of proNGF and sortilin was increased in aging rodent basal forebrain and sympathetic neurons [53].

Despite these important roles of sortilin in the CNS, the detailed molecular mechanisms of sortilin expression remain elusive. Saadipour *et al.* recently demonstrated that amyloid- β_{42} treatment of SH-SY5Y human neuroblastoma cells significantly induced sortilin expression, which is mediated by Rho-associated protein kinase (ROCK) [54]. The present study showed significant AMPK activation and sortilin reduction in the GF compared to the HG condition (Fig. 4A). In addition to this correlation, the present pharmacological experiments also suggested an involvement of AMPK activity in sortilin expression (Fig. 4C,D). Coincidentally, several reports indicated that AMPK activation has negative effects on RhoA-ROCK signaling in vascular smooth-muscle cells [55,56]. Further studies may reveal the precise molecular mechanism(s) of neuronal sortilin expression, with potentially strong impact on neuronal fate.

Conclusion

Because PGRN haploinsufficiency is a crucial factor in some types of neurodegenerative disease, studies of the expressional control of PGRN are extremely important. Although further investigation is required, the

present study suggested that decreased glucose levels around PC12 cells can modify PGRN properties via two distinct mechanisms: p38-dependent induction of *PGRN* expression, and reduction of sortilin levels, controlling AMPK-dependent PGRN endocytosis. Taken together with the fact that PGRN has a neuroprotective action on PC12 cells, this integrated system may shield cell survival from detrimental stress such as glucose deprivation.

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Author contributions

KIK and TN mainly constructed the concept for this research and designed the experiments. HK, NM, JL, and MN helped design and implement the study. KIK, YI, MK, SK, and HS collected, processed, and analyzed data, and also contributed to the literature search. TN wrote the manuscript, with critical revision for important intellectual content from HK, NM, JL, and MN.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article: **Fig. S1.** PGRN pretreatment attenuates H₂O₂-induced PC12 cell death.