

1 **Ginsenoside Rg1 defends PC-12 cells against hydrogen peroxide-caused damage**
2 **via up-regulation of miR-216a-5p**

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4 **Running title:** Protective mechanism of ginsenoside Rg1

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19 **Abstract**

20 **Background:** Spinal cord injury (SCI) is a destructive trauma accompanying with
21 local injury, half of which cause chronic paralysis. Ginsenoside Rg1 exerts
22 anti-apoptosis and anti-autophagy properties. Therefore, our goal was to study the
23 protective mechanism of Rg1 in attenuating cell injury.

24 **Methods:** MiR-216a-5p inhibitor was transfected into PC-12 cells, then cells were
25 pre-treated by Rg1 and treated with 300 μ M hydrogen peroxide (H_2O_2) for 24 h.
26 CCK-8 and apoptosis experiments were done to test cell activity and apoptosis
27 respectively. Expression of miR-216a-5p and cell damage relative factors was tested
28 via qRT-PCR and western blot experiments, respectively.

29 **Results:** H_2O_2 induced cell activity suppression, apoptosis and autophagy well at the
30 concentration of 300 μ M, leading cell injury. Rg1 could attenuate cell injury induced
31 by H_2O_2 at the working concentration of 200 μ M that it elevated cell activity,
32 attenuated apoptosis and autophagy and activated PI3K/AKT and AMPK signal
33 pathways. Further, miR-216a-5p was up-regulated by Rg1. Rg1 played its role in
34 relieving cell injury by positively regulating miR-216a-5p.

35 **Conclusion:** Our study demonstrated that Rg1 attenuated H_2O_2 -caused cell injury
36 through positively regulated miR-216a-5p.

37 **Key words:** ginsenoside Rg1, miR-216a-5p, cell injury

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40 **Introduction**

41 Spinal cord injury (SCI), a common and destructive trauma (Hyun and Kim, 2010), is
42 mainly caused by external forces such as lateral bending, excessive stretching,
43 rotation, axial load and excessive bending, resulting in motor dysfunction, paralysis
44 and other symptoms (van den Berg et al., 2010). Because of the limited therapy
45 selection, the administration and care of SCI patients places a heavy burden on
46 patients and caregivers. Of particular note, more than 60% of damages occur at the
47 cervical level (Cripps et al., 2011), and the lifetime care costs are about at \$1.1-\$4.2
48 million per patient (Krueger et al., 2013). So, the precaution, curing and recovery of
49 SCI are a major topic in the medical area. SCI relates two different stages of tissue
50 injury, called primary and secondary hurt (Badner et al., 2019). Local tissue injury is
51 caused by SCI and is important in secondary hurt in SCI (Fu et al., 2018), leading to
52 apoptosis with loss of neurological roles. So, mechanism study of local injury after
53 SCI is highly significant for curing SCI.

54 Ginsenosides, considering as one of the main pharmacological active ingredients of
55 ginseng, is a steroid compound (Xiang et al., 2008). Ginsenoside contains the
56 Panaxatriol (Rg1, Rg2, Re and Rf) and Panaxadiol (Rb1, Rb2, Rc and Rd) classes
57 (Zhang et al., 2012a). Many beneficial effects of Rg1 have been proved in disorders
58 such as hypertension (Chen et al., 2012), hypoxia/reoxygenation (Zhang et al., 2012b),
59 Alzheimer's disease (Huang et al., 2012) etc. Importantly, it has been reported that
60 Rg1 exerts roles in inhibiting cell apoptosis, thereby exhibiting notable
61 cardioprotective effects against I/R damage through a variety of mechanisms (Lee and
62 Kim, 2014). Besides, Rg1 counteracts the aging of endothelial progenitor cells (Shi et
63 al., 2011) and human fibroblasts (Zhou et al., 2012) and exerts a notable influence in
64 suppressing cardiomyocytes and renal tubular cells' autophagy (Mao et al., 2016). The
65 influence of Rg1 in local injury after SCI still has been unknown yet.

66 MicroRNAs (miRNAs), short (22 nucleotides in length) non-coding RNAs, involve
67 in many biological processes (Jiang and Chen, 2012), such as differentiation of
68 ordinary tissues and are important in the pathogenesis of lots of human cancers
69 (Taucher et al., 2016). MiR-216a-5p, known as an oncogene, involved in the
70 progression of many cancer subtypes (Chen et al., 2018). Chen *et al.* has proved that
71 miR-216a-5p elevates cell proliferation, activity and motility, and inhibits apoptosis
72 (Chen et al., 2018). This finding demonstrates that miR-216a-5p has a positive effect
73 on cell viability and anti-apoptosis. So it could be interesting to investigate if exerts

74 regulation relation of miR-216a-5p and Rg1 in cell injury after SCI. Based on the
75 above questions and guesses, we probed mechanism of Rg1 against H₂O₂-caused cell
76 damage in PC-12 cells.

77 **Materials and Methods**

78 *Cell*

79 PC-12 cells were bought form Kunming Institute of Zoology (Kunming, China) in
80 this whole study. Seed cells at a denseness of 1×10^4 cells/ml in Dulbecco's Modied
81 Eagle Medium (DMEM)/F-12 medium (Gibco, Carlsbad, CA, USA) adding with 10%
82 fetal bovine serum (FBS, Gibco), 100 µg/ml streptomycin and 100 U/ml penicillin
83 (Gibco). Cells were kept in a wet incubator carried 5% CO₂ and 95% air at 37°C.
84 Change fresh medium every day. Ginsenoside Rg1 (analysis level of 97% pureness)
85 was bought from Sigma-Aldrich (St. Louis, MO, USA), solubled in ethanol and stored
86 at -20°C. Pre-treatment of cells with Rg1 for 1 h, and then were treated with a series
87 of consistences of hydrogen peroxide (H₂O₂) for 24 h.

88 *CCK-8 experiment*

89 A Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Gaithersburg, MD,
90 USA) was to test cell activity. Seed cells in 96-well plate with 5000 cells/well, and
91 then add CCK-8 solution, keep cells in a wet environment carried 95% air and 5%
92 CO₂ for 1 h at 37°C. Absorbance was tested at 450 nm via a Microplate Reader
93 (Bio-Rad, Hercules, CA, USA).

94 *Apoptosis experiment*

95 Apoptosis analysis was done through propidium iodide (PI) and fluorescein
96 isothiocyanate (FITC)-conjugated Annexin V staining (BD Pharmingen, San Diego,
97 CA, USA). Cells were cleaned in phosphatebuffered saline (PBS) for three times and
98 stained in PI/FITC-Annexin V with 50 µg/ml RNase A (Sigma-Aldrich). Keep cells in
99 dark processing at the room temperature for 1 h. Flow cytometry analysis was made
100 through FACS can (Beckman Coulter, Fullerton, CA, USA). Data was analyzed via
101 FlowJo software (Tree Star Software, San Carlos, California, USA).

102 *Transfection*

103 MiR-216a-5p inhibitor and its relative NC were compounded by Life Technologies
104 Corporation (Carlsbad, CA, USA) and transferred into cells. Transfection was done
105 following the Lipofectamine 3000 reagent (Life Technologies Corporation). 48 h
106 post-transfection was regarded as harvest moment in following assays.

107 *qRT-PCR*

108 Overall RNA was extracted through Trizol reagent (Life Technologies Corporation)
109 and handled with DNaseI (Promega, Madison, WI, USA). Taqman MicroRNA
110 Reverse Transcription Kit and Taqman Universal Master Mix II with the TaqMan
111 MicroRNA Assay (Applied Biosystems, Foster City, CA, USA) were to test
112 miR-216a-5p expression. U6 was taken as inside comparison.

113 ***Western Blot***

114 Overall protein was extracted through RIPA lysis buffer (Beyotime Biotechnology,
115 Shanghai, China) with protease inhibitors (Roche, Basel, Switzerland), and then
116 quantified through BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). A Bio-Rad
117 Bis-Tris Gel system was taken to build up a western blot system. Primary antibodies
118 specific against Bax (ab32503, Abcam, Cambridge, MA, USA), pro-caspase-3
119 (ab183179), cleaved-caspase-3 (ab49822), pro-PARP (ab32064), cleaved-PARP
120 (ab4830), β -actin (ab8226), beclin-1 (ab62557), p62 (ab56416), LC3-I and LC3-II
121 (ab48394), t-PI3K (ab140307), p-PI3K (ab182651), t-AKT (ab179463), p-AKT
122 (ab38499), t-AMPK (ab131512) and p-AMPK (ab23875) were readied in 5%
123 blocking buffer. Primary antibody was cultured with membrane at 4°C all the night,
124 then washing and incubating with secondary antibody, marking by horseradish
125 peroxidase for 1 h at room temperature. Then the Polyvinylidene Difluoride (PVDF)
126 membrane taken along blots and antibodies were transferred into the Bio-Rad
127 ChemiDoc™ XRS system, adding 200 μ l Immobilon Western Chemiluminescent
128 HRP Substrate (Millipore, MA, USA) to shroud film surface. At last, semaphores
129 were seized and strength of strip was quantified via Image Lab™ Software (Bio-Rad).

130 ***Statistical analysis***

131 All assays were duplicated for 3 times. Our consequences of multiplex assays are
132 revealed as mean \pm SD. Statistical analysis was done via Graphpad Prism 6.0
133 (GraphPad Software Inc., La Jolla, CA, USA). *P*-values were counted via a one-way
134 analysis of variance (ANOVA). *P*-value of < 0.05 indicated statistical significant data.

135 **Results**

136 ***Rgl extenuated H₂O₂-induced cell activity suppression and cell apoptosis***

137 PC-12 cells were treated in various H₂O₂ consistences. From **Figure 1A**, we found
138 that H₂O₂ had notably inhibiting effect on cell viability when the concentration was
139 100 ($P < 0.05$), 200 ($P < 0.05$), 300 ($P < 0.01$), 400 ($P < 0.01$) and 500 μ M ($P <$
140 0.001). We chose 300 μ M as the working concentration in the later assays because this
141 was cell viability semi-lethal concentration. Besides, we tested effect of H₂O₂ on cell

142 apoptosis. We found that apoptosis was notably increased by H₂O₂ ($P < 0.001$, **Figure**
143 **1B**). Similarly, apoptosis relative factors (Bax, cleaved-caspase-3 and
144 cleaved-caspase-PARP) were obviously enhanced through H₂O₂ (**Figure 1C**), and
145 standards of these factors were notably raised (all $P < 0.01$, **Figure 1D**). We got that
146 H₂O₂ caused cell activity suppression and apoptosis.

147 For function of Rg1, following experimental results were clear. As shown in **Figure**
148 **1E**, there was no effect on cell viability by Rg1. We found that H₂O₂ could notably
149 reduce cell viability ($P < 0.01$), whereas Rg1 could notably attenuate this reduction at
150 200, 300 and 400 μ M (all $P < 0.05$). We chose 200 μ M as the working concentration
151 in the following experiments because this is the concentration when cell viability was
152 half restored. Besides, for cell apoptosis, we found that Rg1 attenuated apoptosis
153 induced by H₂O₂ ($P < 0.01$, **Figure 1G**). Similarly, **Figure 1H** revealed that
154 expression of apoptosis relative factors was weakened by Rg1 compared with H₂O₂
155 group. Levels of these factors were raised through H₂O₂ ($P < 0.01$, $P < 0.001$ and $P <$
156 0.01), whereas Rg1 could decrease their levels (all $P < 0.05$, **Figure 1I**). So we got
157 that Rg1 attenuated cell activity suppression and apoptosis induced by H₂O₂.

158 ***Rg1 extenuated autophagy induced by H₂O₂***

159 For autophagy, we tested three autophagy relative factors. Beclin-1 is autophagy gene
160 and its overexpression can stimulate autophagy (Yue et al., 2003). Accumulation of
161 p62 is a notable phenotype of autophagy-deficient tumor cells (Mathew et al., 2009).
162 LC3-II is a marker for mature autophagosomes. Autophagy could be analyzed by
163 testing the conversion of the autophagosome marker LC3-I to LC3-II (Wu et al.,
164 2010). According to our results, **Figure 2A** showed the enhancement of beclin-1 and
165 LC3-II/LC3-I through H₂O₂, while Rg1 could weaken this enhancement. Expression
166 of p62 was weakened by H₂O₂, while Rg1 could eliminate this mitigation (**Figure 2A**).
167 Besides, **Figure 2B** revealed the notable addition of beclin-1 and
168 LC3-II/LC3-I through H₂O₂ ($P < 0.01$ and $P < 0.001$), whereas were opposite by
169 adding of Rg1 ($P < 0.05$ and $P < 0.01$). p62 expression was notably weakened
170 through H₂O₂ ($P < 0.05$), whereas was increased by the adding of Rg1 ($P < 0.05$,
171 **Figure 2B**). So we got that Rg1 could attenuate H₂O₂-induced autophagy.

172 ***Rg1 positively regulated miR-216a-5p***

173 From **Figure 3**, qRT-PCR assay indicated that miR-216a-5p was notably
174 down-regulated after H₂O₂ treatment ($P < 0.05$). But, it was specifically up-regulated
175 by adding Rg1 ($P < 0.01$). So we got that Rg1 up-regulated miR-216a-5p.

176 ***Rg1* attenuated cell activity suppression, apoptosis and autophagy induced by H₂O₂**
177 ***through up-regulating miR-216a-5p***

178 qRT-PCR revealed that miR-216a-5p expression was notably suppressed after
179 miR-216a-5p inhibitor transfection ($P < 0.01$, **Figure 4A**). Cell viability was notably
180 aggravated by Rg1 contrast with H₂O₂ set ($P < 0.05$), whereas was notably alleviated
181 when treated with Rg1 plus miR-216a-5p inhibitor ($P < 0.05$, **Figure 4B**). This result
182 indicated that Rg1 attenuated H₂O₂-induced cell activity suppression by up-regulating
183 miR-216a-5p. Besides, cell apoptosis was notably decreased by Rg1 contrast with
184 H₂O₂ set ($P < 0.01$), whereas was notably increased when treated with Rg1 plus
185 miR-216a-5p inhibitor ($P < 0.01$, **Figure 4C**). **Figure 4D-E** further indicated that
186 levels of apoptosis relative factors were notably decreased through Rg1 contrast with
187 H₂O₂ set ($P < 0.05$, $P < 0.01$ and $P < 0.01$), whereas were raised in Rg1-treated cells
188 with miR-216a-5p inhibitor ($P < 0.05$, $P < 0.01$ and $P < 0.05$). So we got that Rg1
189 reduced H₂O₂-caused apoptosis by up-regulating miR-216a-5p. Additionally, **Figure**
190 **4F-G** revealed the notable attenuation of beclin-1 and LC3-II/LC3-I ($P < 0.01$ and P
191 < 0.001) by Rg1 and level of p62 was increased ($P < 0.05$) by Rg1 contrast with
192 H₂O₂ set. However, beclin-1 and LC3-II/LC3-I were notably enhanced (both $P < 0.05$)
193 and level of p62 was notably decreased when treated with Rg1 plus miR-216a-5p
194 inhibitor ($P < 0.05$). So we got that Rg1 attenuated H₂O₂-induced autophagy by
195 up-regulating miR-216a-5p.

196 ***Signal pathway***

197 To further study the mechanism of Rg1, we focused on PI3K/AKT and AMPK signal
198 pathways. **Figure 5A-B** indicated the notable addition levels of p-PI3K and p-AKT
199 through Rg1 contrast with H₂O₂ set ($P < 0.01$ and $P < 0.05$), whereas were notably
200 alleviated in Rg1-treated cells with miR-216a-5p inhibitor ($P < 0.01$ and $P < 0.05$).
201 Besides, **Figure 5C-D** revealed that level of p-AMPK was notably aggravated by Rg1
202 compared with H₂O₂ group ($P < 0.01$), whereas was notably alleviated in Rg1-treated
203 cells with miR-216a-5p inhibitor ($P < 0.01$). These results indicated that Rg1 elevated
204 PI3K/AKT and AMPK pathways via positively modulating miR-216a-5p.

205 **Discussion**

206 SCI is a life-changing event. Recently, there is no effective treatment method to
207 resume the functions of SCI patients. The complexity of SCI pathophysiology poses a
208 huge challenge for researchers and clinicians seeking to develop therapeutic
209 interventions (Bareyre, 2019). Local injury is the main event of secondary injury in

210 SCI, eventually leading to apoptosis and ultimately loss of neurological function
211 (Genovese et al., 2009). Therefore, defending cells against local injury or relieving
212 this injury can be an effective method to cure SCI. Rg1, an active component of
213 ginsenosides, has been proved to exert positive effect on anti-apoptosis (Zu et al.,
214 2016). Cell damage has been reported to be induced by H₂O₂ in cardiomyocytes
215 (Zeng et al., 2019) and neural cells (Chen et al., 2016). Therefore, our study also
216 found that H₂O₂ was a mediator capable of inducing damage in PC-12 cells, including
217 activity suppression, promotion of autophagy and apoptosis. Our study firstly
218 researched the attenuated mechanism of Rg1 in H₂O₂-caused damage in PC-12 cells.
219 Rg1 was effective in defending PC-12 cells against H₂O₂-caused damage. Rg1 could
220 extenuate H₂O₂-induced cell activity suppression, apoptosis and autophagy, and active
221 PI3K/AKT and AMPK pathways via positively regulating miR-216a-5p. Our results
222 indicated that Rg1 may be an effective treatment of curing SCI.

223 Ginsenoside Rg1, the main bioactive ingredient in ginseng, has been proved to exert
224 low toxicity that there was no change on cell viability and proliferation (Li et al.,
225 2017). Much evidence indicates that Rg1 exerts beneficial effects, like anti-aging
226 properties (Zhu et al., 2014). As we all know, Bax may control mitochondrial
227 permeability transition and promote releasing cytochrome c, ultimately triggering the
228 activation of caspases, leading to apoptosis (Li et al., 2017). Consistently, in our study,
229 treatment of Rg1 reduced the level of Bax, at the same time, cleaved-caspase-3 and
230 cleaved-caspase-PARP were weakened. These observations verified the anti-apoptosis
231 function of Rg1. Additionally, autophagy is an important cellular process where
232 cytoplasmic components are digested by lysosomes to keep cell homeostasis and
233 energy production (Ravikumar et al., 2010). Rg1 has a notable pharmacological
234 influence in suppressing autophagy (Mao et al., 2016). Our study were consistent with
235 the report that Rg1 strongly inhibited autophagic factor (beclin-1 and LC3-II/LC3-I),
236 and counteracted attenuation of p62 induced by H₂O₂, leading to inhibiting
237 H₂O₂-induced autophagy in PC-12 cells. Consistently, our study indicated that Rg1
238 could counteract inhibition of cell activity induced by H₂O₂ and attenuate cell
239 apoptosis and autophagy, suggesting the anti-oxidant and anti-autophagy functions of
240 Rg1 in SCI.

241 To further study the mechanism of Rg1, we turn our attention to miRNA. MiRNA is
242 important in cell growth, like proliferation and apoptosis (Ameres and Zamore, 2013).
243 MiR-216a-5p, acknowledged as an oncogenic gene, is involved in tumorigenesis and

244 development of human cancers (Liu et al., 2018). MiR-216a-5p significantly elevated
245 cell activity and reduced apoptosis in H₂O₂-caused 16 HBE cells of Asthma,
246 suggesting that miR-216a-5p could regulate H₂O₂-caused damage (Chaoyang et al.,
247 2019). Besides, of interest, beclin-1 was the latent mark of miR-216-5p, which could
248 inhibit ox-LDL-induced autophagy in human umbilical vein endothelial cells
249 (HUVECs) through modulating levels of intracellular beclin-1 (Menghini et al., 2014).
250 These reports indicate that miR-216a-5p not only elevates activity, suppresses
251 apoptosis, but also inhibits autophagy, suggesting that miR-216a-5p may reduce
252 H₂O₂-caused cell damage. Also, functions of miR-216a-5p are similar to those of Rg1.
253 So it is worth to investigate if exerts a relation of Rg1 and miR-216a-5p. For the first
254 time, our study found the regulation relationship between Rg1 and miR-216a-5p. We
255 got that Rg1 could up-regulate miR-216a-5p to attenuate cell injury induced by H₂O₂.
256 This finding is a major discovery in SCI research.

257 Furthermore, the biological process is inseparable from the regulation of signal
258 pathways. It has been proved that AMPK/PI3K/AKT pathways are key coordinator
259 protecting cells from oxidative and inflammatory damage (Lv et al., 2017). PI3K was
260 reported to be related to many cellular functions, like proliferation and apoptosis
261 (Rasul et al., 2012). AKT is a key downstream effector of PI3K and exhibits
262 anti-apoptotic effects (Zheng et al., 2015). AMPK is present in metabolically related
263 organs. Cellular metabolism stimulation like cell stress can active it (Zheng et al.,
264 2015). Moreover, Lin *et al.* found that the formation of autophagosomes was
265 accompanied by inhibition of the PI3K/AKT and AMPK signal pathways. This
266 finding indicated that there was negative regulation between these two pathways and
267 autophagy. Therefore, the above findings suggested that there might be positive
268 effects of these two pathways on H₂O₂-caused cell damage. It is worth noting that our
269 study verified this suppose. We firstly build up the relation among Rg1, miR-216a-5p
270 and AMPK/PI3K/AKT pathways that Rg1 activated PI3K/AKT and AMPK signal
271 pathways through positively modulating miR-216a-5p to reduce cell damage. This
272 regulation mechanism provides a theoretical basis for attenuating cell injury after SCI.

273 **Conclusion**

274 Our study firstly reported the underlying effects and mechanism of Rg1 in cell injury
275 of SCI. We demonstrated that Rg1 could up-regulate miR-216a-5p, attenuate cell
276 activity suppression, apoptosis and autophagy, and active PI3K/AKT and AMPK
277 signal pathways to decrease H₂O₂-caused damage in PC-12 cells. Because local cell

278 injury significantly aggravates SCI, we propose that Rg1, an effective biological
279 macromolecular, may supply a novel therapeutic approach for curing SCI.

280 **Conflict of Interest statement**

281 The authors declare that there are no conflicts of interest.

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287 **Availability of data and materials**

288 The dataset(s) supporting the conclusions of this article is(are) included within the
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290

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390

391 **Figure legends**

392 **Figure 1** Influence of Rg1 in cell activity and apoptosis caused by H₂O₂ in PC-12 cell.

393 (A) Cell viability was tested under diverse consistences of H₂O₂ (0, 100, 200, 300,
394 400 and 500 μM). 300 μM was chose in the following experiments. (B) Cell apoptosis
395 treated by H₂O₂ was tested via flow cytometry. (C) Apoptosis relative elements
396 expression was tested via western blot. (D) Level of apoptosis relative factors was
397 tested via western blot quantitative. (E) Cell viability was tested via CCK-8 by Rg1.
398 (F) Rg1 attenuated H₂O₂-induced suppression of cell viability. (G) Rg1 attenuated
399 H₂O₂-induced cell apoptosis. (H) Expression of apoptosis relative factors was tested
400 via western blot. (I) Apoptosis relative elements standards were detected via western
401 blot quantitative. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ contrast with control and
402 the indicated set.

403 **Figure 2** Influence of Rg1 in autophagy caused by H₂O₂. (A) Standards of autophagy
404 relative factors were tested via western blot. (B) Standards of autophagy relative
405 factors were tested via western blot quantitative. * $P < 0.05$, ** $P < 0.01$ and *** $P <$
406 0.001 contrast with indicated set.

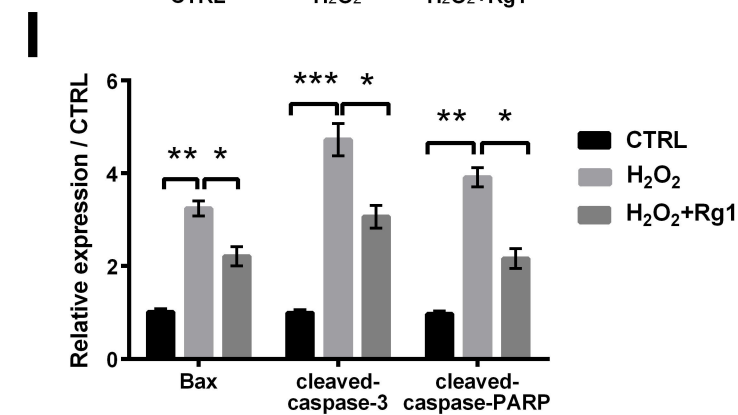
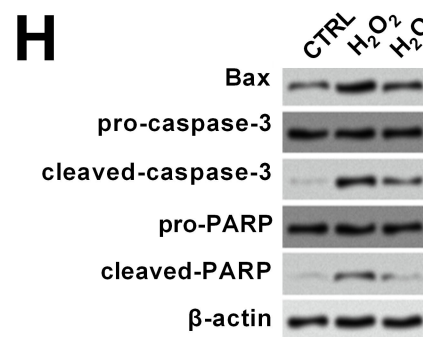
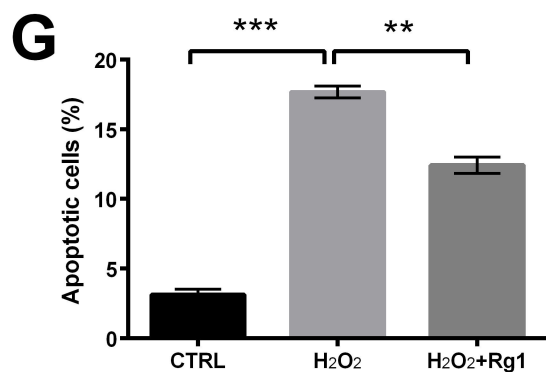
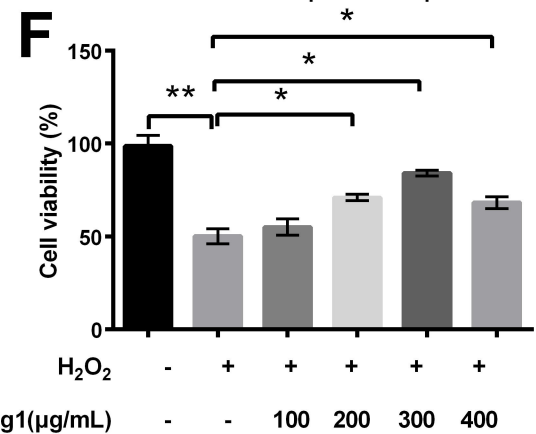
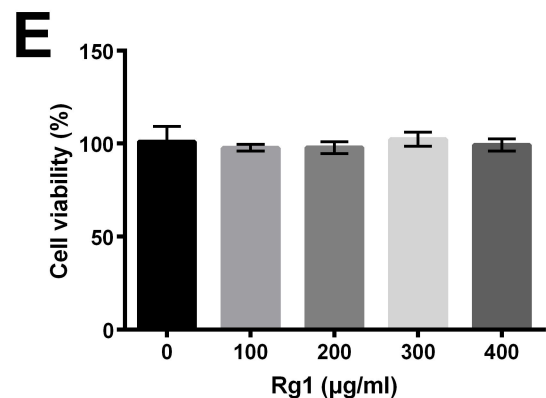
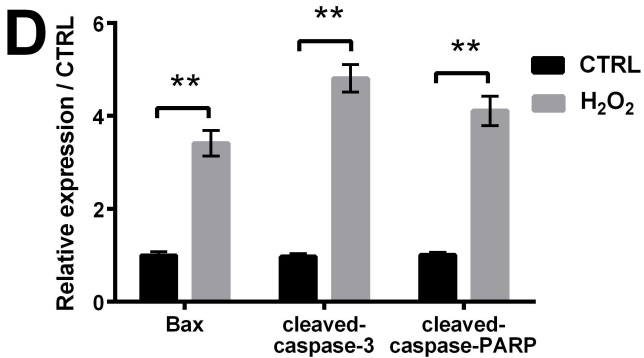
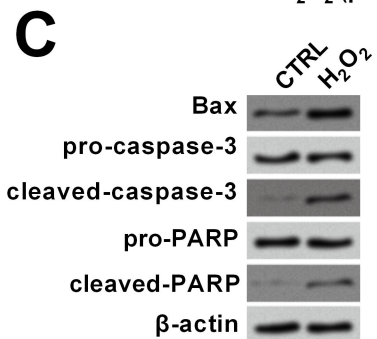
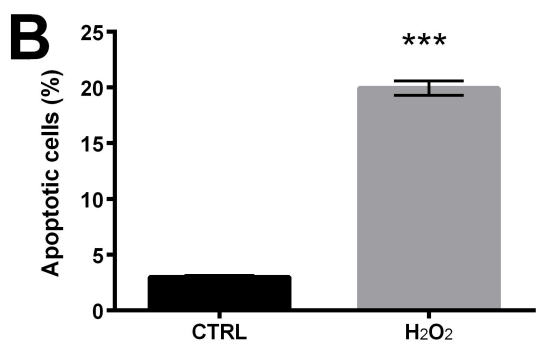
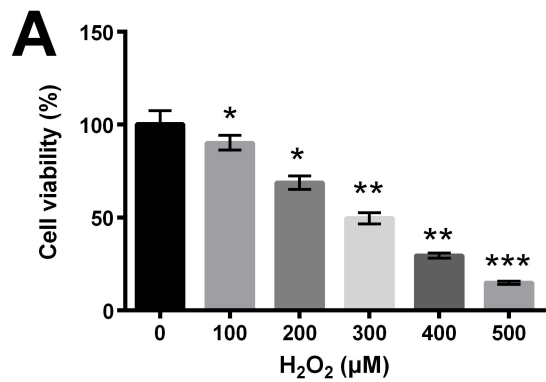
407 **Figure 3** MiR-216a-5p was up-regulated by Rg1. mRNA standard of miR-216a-5p
408 was tested via qRT-PCR. * $P < 0.05$ and ** $P < 0.01$ contrast with indicated set.

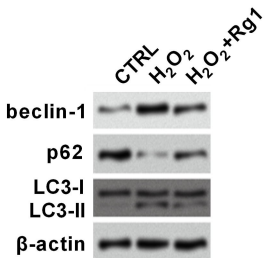
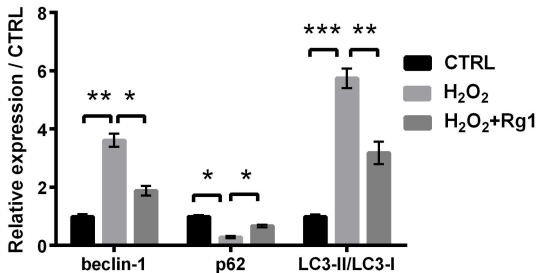
409 **Figure 4** Protective effects of Rg1 via up-regulating miR-216a-5p after transfected
410 with Rg1 plus miR-216a-5p and relative NC in PC-12 cells. (A) mRNA standard of
411 miR-216a-5p was tested via qRT-PCR after miR-216a-5p inhibitor transfection. (B)
412 Cell activity was tested via CCK-8. (C) Apoptosis was tested via flow cytometry. (D)
413 Expression of apoptosis relative factors was tested via western blot. (E) Level of
414 apoptosis relative factors was tested via western blot quantitative. (F) Standards of
415 autophagy relative factors were tested via western blot. (G) Standards of autophagy
416 relative factors were tested via western blot quantitative. * $P < 0.05$, ** $P < 0.01$ and
417 *** $P < 0.001$ contrast with indicated set.

418 **Figure 5** Rg1 elevated PI3K/AKT and AMPK signal pathways through positively
419 regulating miR-216a-5p after transfected with Rg1 plus miR-216a-5p and relative NC
420 in PC-12 cells. (A) Expression of PI3K/AKT pathway relative factors was tested via

421 western blot. (B) Standards of relative proteins were tested via western blot
422 quantitative. (C) Expression of AMPK pathway relative factors was tested via western
423 blot. (D) Level of AMPK pathway related proteins were detected via western blot
424 quantitative. * $P < 0.05$ and ** $P < 0.01$ contrast with indicated set.

425



A**B**

miR-216a-5p

