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Compound 13, an α1-selective small molecule activator of AMPK, inhibits *Helicobacter pylori*-induced oxidative stresses and gastric epithelial cell apoptosis

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ABSTRACT

Half of the world's population experiences *Helicobacter pylori* (*H. pylori*) infection, which is a main cause of gastritis, duodenal and gastric ulcer, and gastric cancers. In the current study, we investigated the potential role of compound 13 (C13), a novel α1-selective small molecule activator of AMP-activated protein kinase (AMPK), against *H. pylori*-induced cytotoxicity in cultured gastric epithelial cells (GECs). We found that C13 induced significant AMPK activation, evidenced by phosphorylation of AMPKα1 and ACC (acetyl-CoA carboxylase), in both primary and transformed GECs. Treatment of C13 inhibited *H. pylori*-induced GEC apoptosis. AMPK activation was required for C13-mediated GEC protection. Inhibition of AMPK kinase activity by the AMPK inhibitor Compound C, or silencing AMPKα1 expression by targeted-shRNAs, alleviated C13-induced GEC protective activities against *H. pylori*. Significantly, C13 inhibited *H. pylori*-induced reactive oxygen species (ROS) production in GECs. C13 induced AMPK-dependent expression of anti-oxidant gene heme oxygenase (HO-1) in GECs. Zinc protoporphyrin (ZnPP) and tin protoporphyrin (SnPP), two HO-1 inhibitors, not only suppressed C13-mediated ROS scavenging activity, but also alleviated its activity in GECs against *H. pylori*. Together, these results indicate that C13 inhibits *H. pylori*-induced ROS production and GEC apoptosis through activating AMPK–HO–1 signaling.

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1. Introduction

Half of the world's populations have *Helicobacter pylori* (*H. pylori*) infection, which is a gram-negative bacterium that selectively colonizes at gastric mucosa [1,2]. *H. pylori* infection could cause gastritis, duodenal and gastric ulcer, and gastric cancers [1,2]. The interactions between the bacterial factors and host cells likely determine the fate of host cells, manifesting as increased proliferation, and either increased or decreased apoptosis [1,2]. *In vivo* and *in vitro* studies have demonstrated that several *H. pylori* virulence factors could induce gastric epithelial cell (GEC) apoptosis [3–5]. The underlying mechanisms of *H. pylori*-induced apoptosis in GECs are not fully understood. Activation of major histocompatibility complex class II [6], p53 [5], Fas/FasL system [7], and tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL) [8] have been proposed. In addition, *H. pylori*-induced GEC apoptosis is often associated with reactive oxygen species (ROS) production [9].

AMP-activated protein kinase (AMPK) is a sensor of cellular energy status [10]. In addition, AMPK activation could also promote cell survival through regulating several key molecules [11]. For example, activated AMPK triggers cytoprotective autophagy induction via directly phosphorylating autophagy kinases (ULK1, Beclin 1, and Vps34). AMPK-activated autophagy then generates new cellular components and energy to help cell survival [12,13]. Jeon et al. showed that stress-activated AMPK has ROS scavenging activity, and inhibits oxidative stress through maintaining nicotinamide adenine dinucleotide phosphate (NADPH) homeostasis [14]. AMPK also activates tuberous sclerosis complex 2 (TSC2) to

Abbreviations: AMPK, AMP-activated protein kinase; ACC, acetyl-CoA carboxylase; C13, compound 13; GECs, gastric epithelial cells; *H. pylori*, *Helicobacter pylori*; HO-1, heme oxygenase; ROS, reactive oxygen species; shRNA, short hairpin RNA; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end; SnPP, tin protoporphyrin; ZnPP, zinc protoporphyrin.

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inhibit mammalian target of rapamycin (mTOR), and promotes cell survival under energy starvation conditions [15,16]. Our previous study identified that *H. pylori*-activated AMPK activation promotes GEC survival [17].

In this study, we investigated the potential role of compound 13 (C13), an α1-selective small molecule activator of AMPK [18], against *H. pylori*-induced GEC apoptosis. Our results show that C13 exerts GEC protective activity against *H. pylori* through activating AMPK-heme oxygenase (HO-1) signaling.

2. Material and methods

2.1. H. pylori culture

H. pylori 26695 [17] was maintained on blood agar plates (Becton Dickinson and Company, Franklin Lakes, NJ). *H. pylori* was added to GECs at a multiplicity of infection (MOI) of 100:1 and co-cultured with GECs for the indicated times.

2.2. GES-1 cell culture

An immortalized human GEC line [17], GES-1 cells were maintained in RPMI 1640 medium supplemented with 10% FBS (Gibco), with necessary antibiotics in a humidified atmosphere of 5% CO_2.

2.3. Human GECs isolation and culture

As previously reported [17], gastric biopsy specimens from the antral gastric mucosa were collected from adult patients undergoing esophagogastroduodenoscopy. The antral gastric mucosa were minced and incubated with 10 mM dithiothreitol and 1 mM EDTA at room temperature for 1 h with vigorous shaking. Cells were filtered through 70-μm nylon mesh, pelleted by centrifugation, and washed with PBS. The GECs were isolated, and resuspended in RPMI 1640 containing 20% FBS with antibiotics. The gastric epithelial population was tested by staining cells using an antibody against cytokeratin (Cell Signaling Tech, Shanghai, China), a specific epithelial marker, and flow cytometry. More than 90% of isolated cells were positive for cytokeratin. A total of 5×10^5 cells were plated in 12-well plates, allowed to adhere for 5 h, and then infected with *H. pylori* or with indicated treatments. All patients enrolled provided individual informed consent with author institutions' approved protocols. The experiments conformed to the principles set out in the Declaration of Helsinki and the NIH Belmont Report.

2.4. Western blots

The lysates (20 μg) from each sample were subjected to electrophoresis through 10% SDS-polyacrylamide gels and transferred onto PVDF membranes (Millipore). The membrane was blocked overnight with 5% nonfat milk at 4 °C and incubated with indicated primary antibody overnight. After three to five washes with PBST, the secondary antibody was added according to standard procedures. The membrane was washed another three times and developed using the ECL system (Amersham Pharmacia, Shanghai, China). Intensity of each band was quantified through ImageJ software, and value was normalized to the corresponding loading.

2.5. Antibodies and reagents

C13 was synthesized by Biyuntian Biotech (Wuxi, China) based on the structure in Ref. [18]. Anti-AMPKα1, AMPKα2, acetyl-CoA carboxylase (ACC), and β-tubulin antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against phospho(p)-AMPKα1 (Thr 172) and p-ACC (Ser 79) were purchased form Cell Signaling Tech. (Denver, MA, USA). Compound C, A769662, tin protoporphyrin (SnPP) and zinc protoporphyrin (ZnPP) were purchased from Sigma Chemicals (Shanghai, China).

2.6. Apoptosis assay

GEC apoptosis was detected by Annexin V Flow cytometry (FACS) assay, Histone-DNA enzyme-linked immunosorbent assay (ELISA) assay or terminal deoxynucleotidyl transferase dUTP nick end (TUNEL) staining assay as described [17].

2.7. MTT assay for GEC survival

GECs were seeded into wells containing 100 μL of the culture medium of a 96-well plate and incubated overnight at 37 °C. After applied treatment, 25 μL of 5 μg/mL MTT (Sigma Chemical Co.,) labeling reagent was added to the designated wells and cells were further incubated at 37 °C for 4 h. The supernatant was removed, and then 150 μL of dimethyl sulfoxide (DMSO) was added. After the plate was incubated at 37 °C for 10 min, the absorbency was measured using a micro ELISA reader (Bio-Tek Instruments, Winooski, VT, USA) at 570 nm.

2.8. Knockdown of AMPKα1 or AMPKα2 by short hairpin RNA (shRNA)

The lentiviral particles containing scramble shRNA (sc-shRNA) or shRNAs of AMPKα1 were synthesized, verified and provided by Kaiji Biotech (Shanghai, China). The hairpin sequences for AMPKα1 were as follows. 5′-GCATAATAAGTCACAGCCAAA-3′ (AMPKα1 shRNA-1) and 5′CTCCAAGACCAGGAAGTCATACAATAGAA-3′(AMPKα1 shRNA-2) (see our previous publication [17]). AMPKα2 shRNA-containing lentiviral particles were obtained from Santa Cruz. GECs were seeded in a 6-well plate in growth medium with 50% confluence. The lentiviral particles (20 μL/ml medium) were added to the cells, after 12 h, the lentiviral particles containing medium was replaced by cell culture medium, and cells were further cultured for another 24 h. Puromycin (Sigma, 0.25 μg/ml) was added to select resistant stable colonies. The expression of AMPKα1, AMPKα2 and β-tubulin (loading) in stable cells was tested by Western blots.

2.9. Reactive oxygen species (ROS) assay

The ROS level was determined by carboxy-H2DCFDA staining assay, which is based on the fact that the nonpolar, nonionic H2-DCFDA crosses cell membranes and is hydrolyzed into non-fluorescent H2-DCF by intracellular esterase. In the presence of ROS, H2-DCF is rapidly oxidized to become highly fluorescent DCF. After applied treatment, GECs were incubated with 1 μM of carboxy-H2-DCFDA at 37 °C for 30 min. Cells ($1*10^6$) were then resuspended in PBS and sent to flow cytometry analysis (BD bioscience).

2.10. Real-time PCR

Total RNA of GECs was extracted through TRIzol reagents (Invitrogen, USA), and reverse transcription was performed using TOYOBO ReverTra Ace-a RT-PCR kit (TOYOBO, Japan) according to the manufacturer's instructions. Real-time PCR was performed on a Bio-Rad IQ5 multicolor detection system. One RNA sample of each preparation was processed without real time-reaction to provide a negative control in subsequent PCR. After amplification, melt curve

analysis was performed to analyze product melting temperature. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was chosen as the reference gene for normalization, and the 2-ΔΔCT (Cycle Threshold) method [19] was applied to quantify HO-1 mRNA fold changes within samples. The primers used for HO-1 were: forward, 5′-AAGATTGCCCAGAAAGCCCTGGAC-3′ and reverse, 5′-AACTGTCGC CACCAGAAAGCTGAG-3′ [20]. The primers used for *GAPDH* were: forward, 5′-GGAGTCAACGGATTTGGTCGTA-3′ and reverse, 5′-GGCAACAATATCCACTTTACCAGAGT-3′ [21].

2.11. Statistical analysis

All statistical analyses were performed using SPSS statistical software (SPSS version 16.0, Chicago, USA). The values were presented as the mean ± SD of three independent experiments. The comparisons among multiple groups were performed using analysis of variance (ANOVA). $^{*}P < 0.05$ was considered statistically significant.

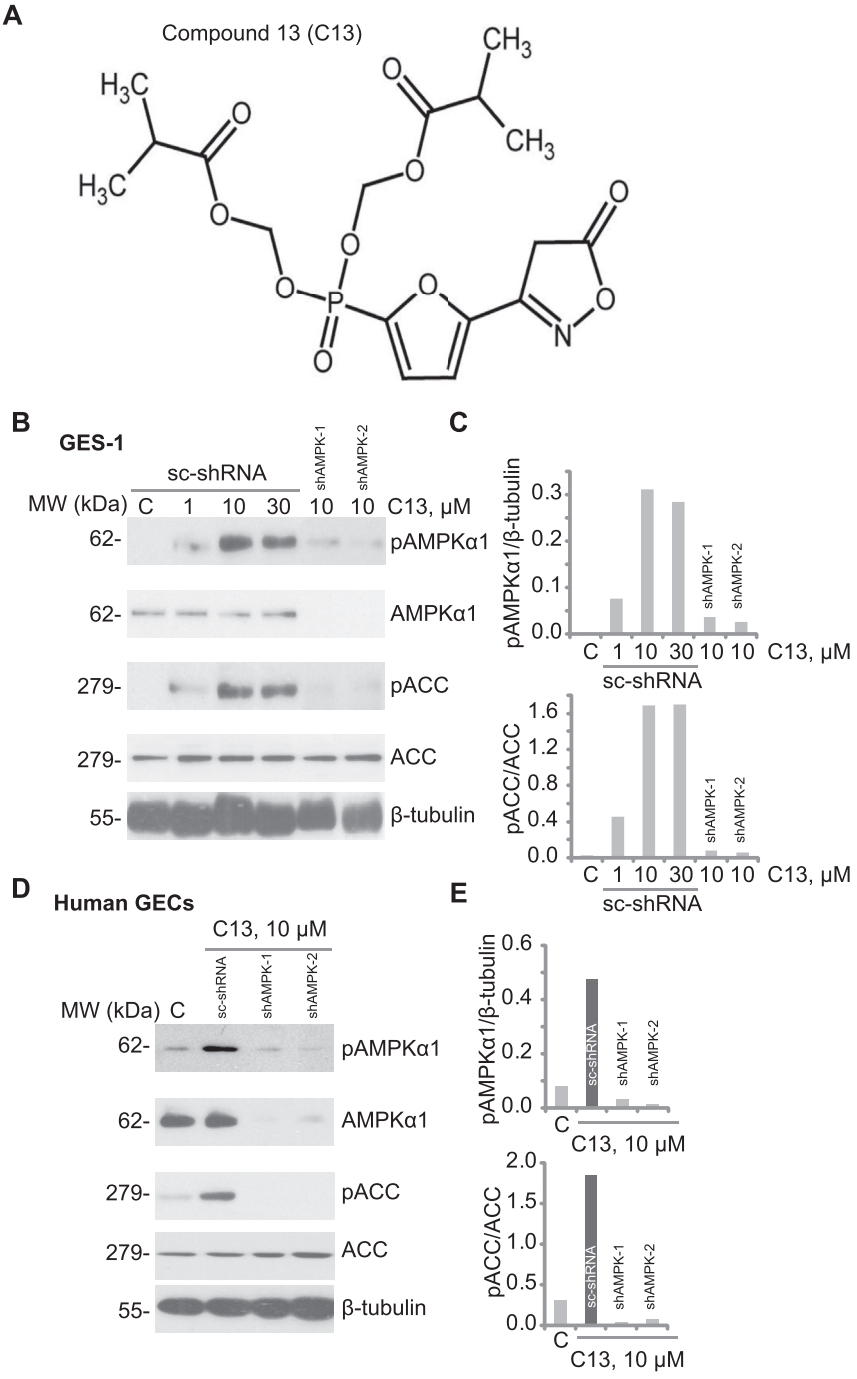

Fig. 1. *C13 activates AMPK in cultured gastric epithelial cells (GECs)*-The structure of compound 13 (C13) (**A**). GES-1 cells (**B** and **C**) or primary human GECs (**D** and **E**), with scramble shRNA ("sc-shRNA") or indicated AMPKα1 shRNAs ("shAMPK-1/-2") lentiviral particles, were stimulated with applied concentration of C13 for 1 h, expression of phospho- (p-) and regular AMPKα1/ACC and β-tubulin (loading control) was tested by Western blots. Phosphorylations of AMPKα1 (vs. β-tubulin) and ACC (vs. regular ACC) were quantified (**C** and **E**). Data in this and all subsequent figures were representative of at least three separate experiments. "C" stands for cell alone (Same for all figures).

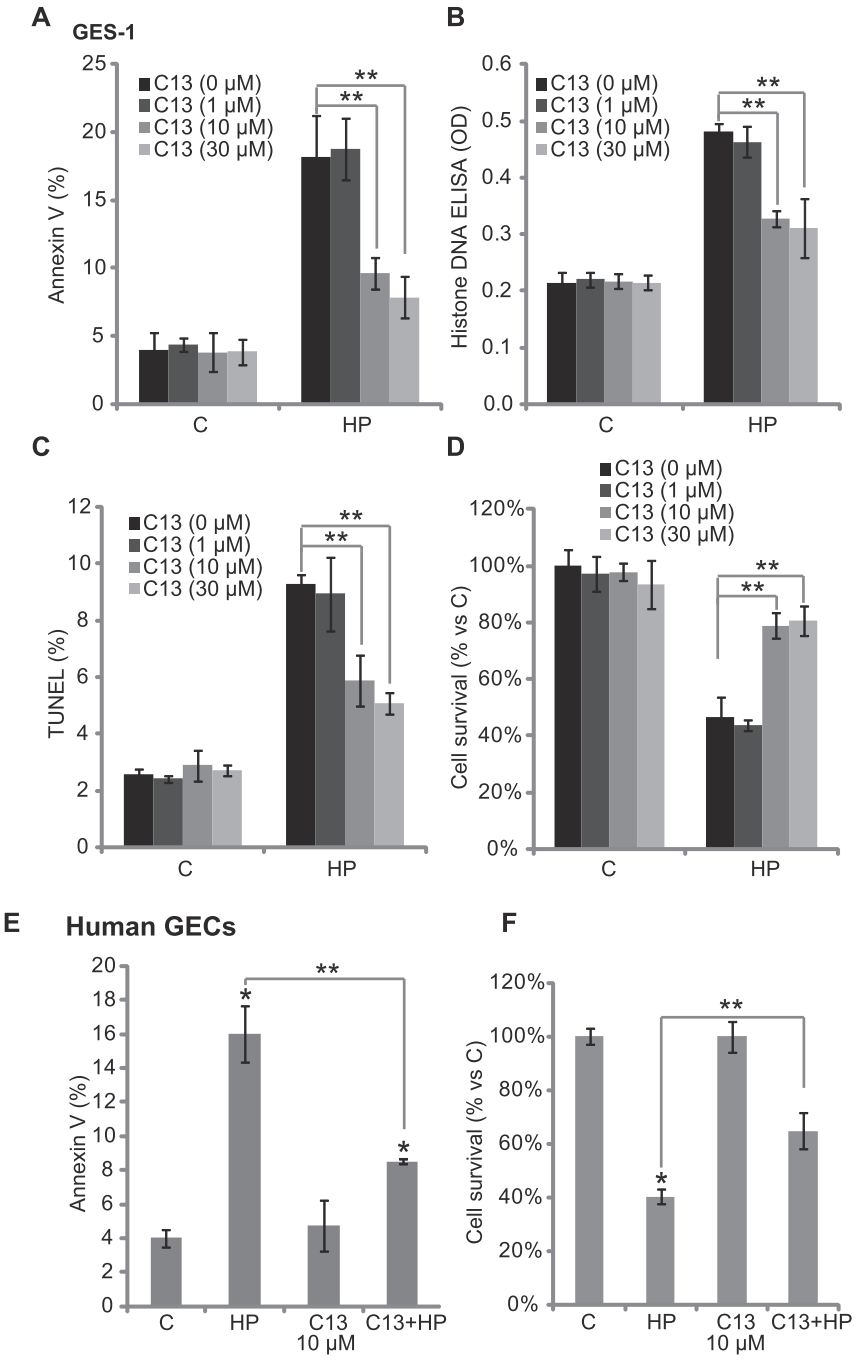

Fig. 2. *C13 alleviates H. pylori-induced GEC apoptosis*-GES-1 cells or primary human GECs, pretreated for 30 min with applied concentration of C13, were infected with the *H. pylori* (HP, bacteria: cells = 100:1) for 24 h, cell apoptosis was tested by Annexin V FACS assay (**A** and **E**), Histone-DNA ELISA assay (**B**, for GEC-1 cells) or TUNEL staining assay (**C**, for GEC-1 cells), cell viability was tested by MTT assay (**D** and **F**). Data in this figure were repeated five times. $^{*}P < 0.05$ vs. "C" group. $^{**}P < 0.05$.

3. Results

3.1. C13-induced AMPK activation requires α1 subunit in cultured gastric epithelial cells (GECs)

Based on the structure of C13 (Fig. 1A) [18], we successfully synthesized this novel AMPK activator. We examined whether GECs were response to this newly synthesized compound. Western blot results in Fig. 1B demonstrated that C13 dose-dependently activated AMPK in cultured GES-1 cells. AMPK activation was evidenced by phosphorylation of AMPKα1 (Thr 172) and ACC (Ser 79) (Fig. 1B and C). Since C13 is an α1-selective small molecule activator of AMPK, it induces activation of AMPK through interaction with α1 subunit [18]. Next, we tested the role of AMPKα1 in C13-mediated AMPK activation in GES-1 cells. ShRNA method was applied to knockdown AMPKα1 expression in GES-1 cells. Two non-overlapping AMPKα1 shRNAs [17] showed high efficiency, resulting in over 90% knockdown efficiency (Fig. 1B). Notably, C13-induced AMPK activation (AMPKα1/ACC phosphorylation) was prevented by AMPKα1 knockdown (Fig. 1B and C). In primary cultured human GECs, C13 treatment similarly induced AMPK activation (Fig. 1D and E). In addition, AMPKα1 silencing by shRNAs

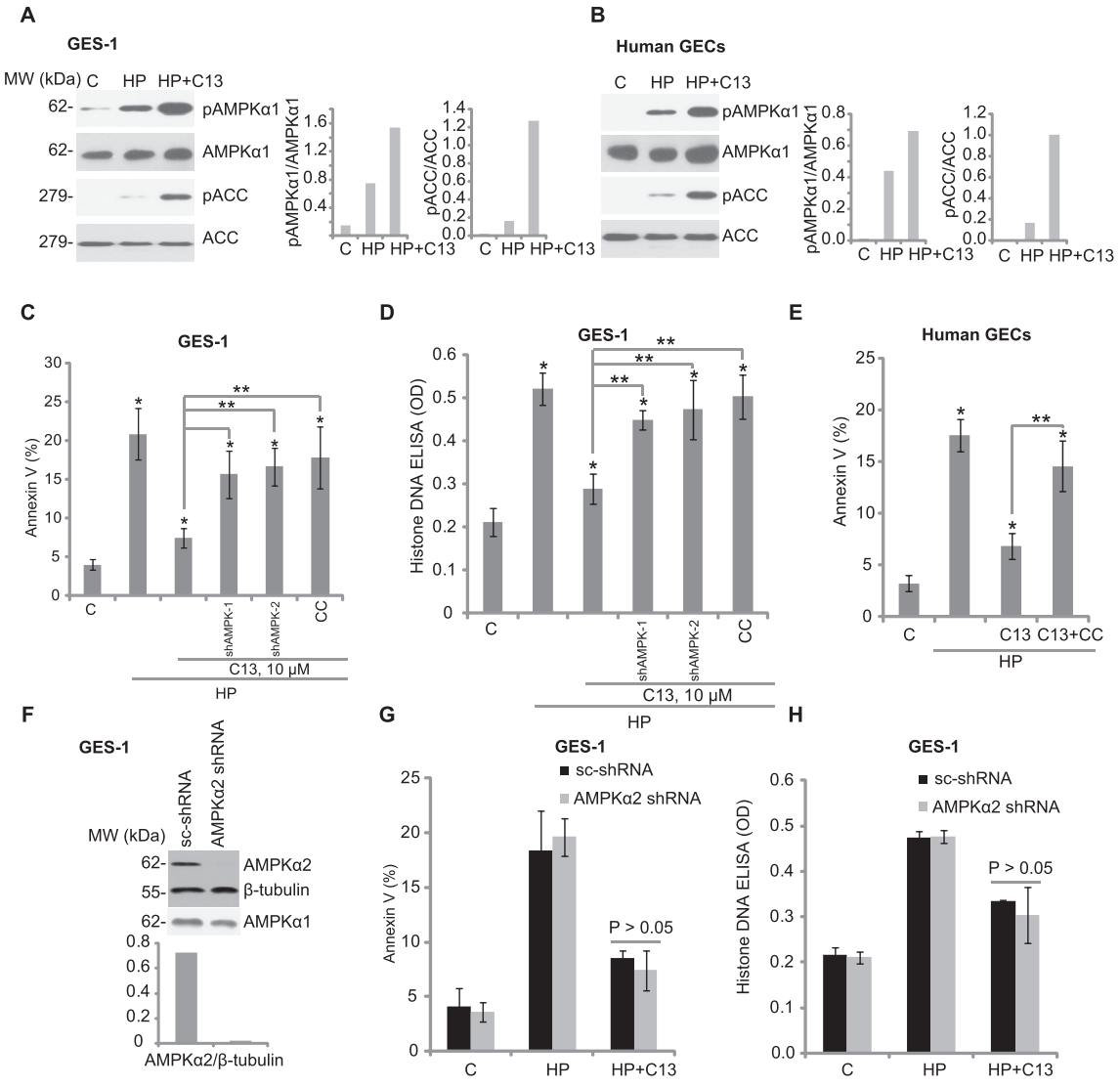

Fig. 3. *AMPK activation is required for C13-mediated anti-H. pylori activities in GECs*-GES-1 cells (**A**) or primary human GECs (**B**), pretreated for 30 min with C13 (10 μM), were infected with the *H. pylori* (HP, bacteria: cells = 100:1) for 3 h, expression of phospho- (p-) and regular AMPKα1/ACC was tested by Western blots. Phosphorylations of AMPKα1 (vs. AMPKα1) and ACC (vs. regular ACC) were quantified (**A** and **B**, right panels). GES-1 cells, with or without AMPKα1 shRNAs ("shAMPK-1/-2") lentiviral particles, were pre-added with C13 (10 μM) or plus Compound C (CC, 10 μM), followed by *H. pylori* (HP, bacteria: cells = 100:1) infection for 24 h, cell apoptosis was tested by Annexin V FACS (**C** and **E**) assay and Histone DNA-ELISA assay (**D**). Stable GES-1 cells, with scramble control shRNA ("sc-shRNA") or AMPKα2 shRNA, were pre-added with C13 (10 μM), followed by *H. pylori* (HP, bacteria: cells = 100:1) infection for 24 h, expression of AMPKα1, AMPKα2 and β-tubulin was shown (**F**), cell apoptosis was tested by Annexin V FACS (**G**) assay and Histone DNA-ELISA assay (**H**). $^{*}P < 0.05$ vs. "C" group. $^{**}P < 0.05$.

inhibited C13-mediated AMPK activation in primary GECs (Fig. 1D and E). Thus, we show that C13 activates AMPK in GECs probably through interacting with α1 subunit.

3.2. C13 alleviates H. pylori-induced GEC apoptosis

Our previous study showed that activation of AMPK by traditional AMPK activators (A769662 and resveratrol), or by overexpression of AMPKα1, inhibited *H. pylori*-induced GEC apoptosis [17]. Next, we examined the activity of C13 on *H. pylori* in cultured GECs. As expected, *H. pylori* induced significant GES-1 cell apoptosis, which was evidenced by three different apoptosis assays, including Annexin V FACS assay (Fig. 2A), Histone DNA ELISA assay (Fig. 2B) and TUNEL staining assay (Fig. 2C). Notably, C13 treatment at 10 μM or 30 μM inhibited *H. pylori*-induced GEC apoptosis (Fig. 2A–C). GES-1 cell viability, tested by MTT assay, was restored by C13 in *H. pylori*-infected GES-1 cells (Fig. 2D). C13 alone at applied concentrations (1–30 μM) had no significant effect on GEC apoptosis or survival (Fig. 2A–D). In primary human GECs, C13 (10 μM) treatment inhibited *H. pylori*-induced apoptosis (Fig. 2E) and viability reduction (Fig. 2F). Together, these results display that C13, the novel AMPK activator, alleviates *H. pylori*-induced GEC apoptosis.

3.3. AMPK activation is required for C13-mediated anti-H. pylori activities in GECs

In consistent with our previous study [17], we found that *H. pylori* induced moderate AMPK activation (AMPKα1/ACC phosphorylation) in GES-1 cells (Fig. 3A) and in primary human GECs (Fig. 3B) [17]. Significantly, co-treatment with C13 further enhanced AMPK activation (Fig. 3A and B). Next, we studied the association between AMPK activation and C13-mediated anti-*H. pylori* activities. Both pharmacological and shRNA strategies were

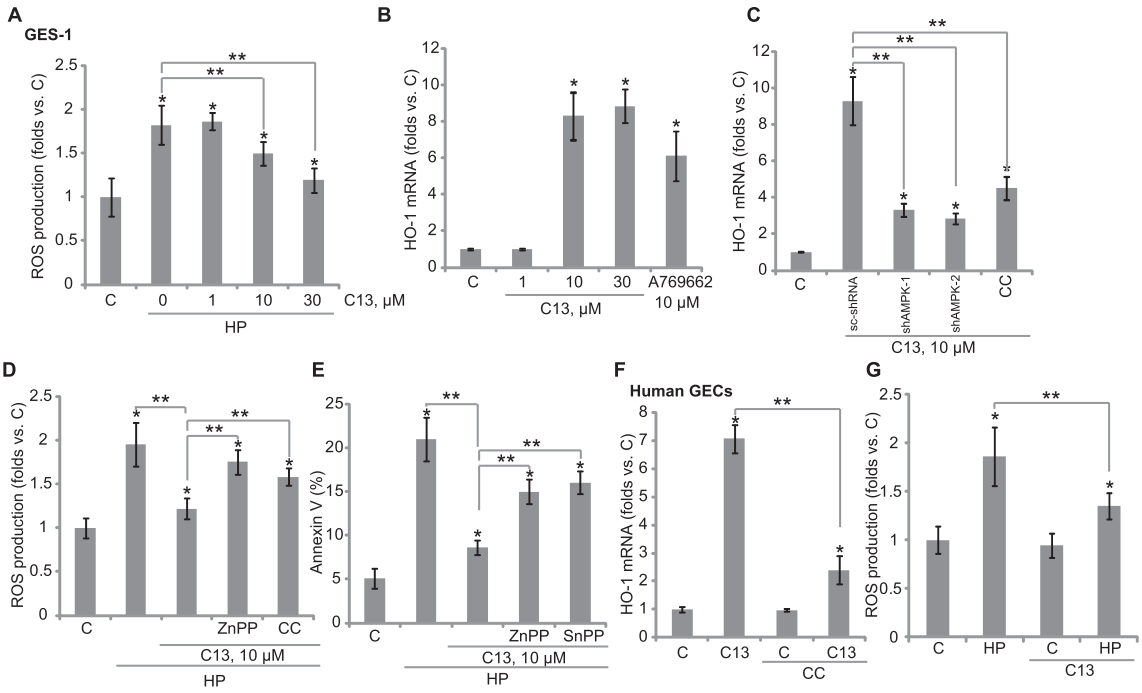

Fig. 4. *C13 inhibits H. pylori-induced ROS production*-GES-1 cells, pretreated for 30 min with applied concentration of C13, were infected with the *H. pylori* (HP, bacteria: cells = 100:1) for 6 h, ROS production was analyzed by FACS assay, their values were normalized to cell only group ("C") (**A**). GES-1 cells were treated with applied concentration of C13 or A769662 (10 μM) for 3 h, relative HO-1 mRNA (vs. GAPDH) was tested by real-time PCR (**B**). GES-1 cells, with or without scramble shRNA ("sc-shRNA") or indicated AMPKα1 shRNAs ("shAMPK-1/-2") lentiviral particles, were treated with C13 (10 μM) or plus Compound C (CC, 10 μM) for 3 h, relative HO-1 mRNA expression (vs. GAPDH) was tested (**C**). GES-1 cells, pre-treated with Compound C (CC, 10 μM), ZnPP (5 μM) or SnPP (5 μM) for 30 min, were stimulated with C13 (10 μM), followed by *H. pylori* (HP, bacteria: cells = 100:1) infection, after 6 h, ROS level was measured (**D**), 24 h after HP infection, cell apoptosis was tested by Annexin V FACS assay (**E**). Human GECs were pre-added with Compound C (CC), followed by C13 stimulation for 3 h, HO-1 mRNA expression was analyzed as described (**F**). Human GECs, pretreated with C13 (10 μM) for 30 m, were infected by *H. pylori* (HP, bacteria: cells = 100:1) for 6 h, ROS production was analyzed (**G**). **P* < 0.05 vs. Group "C". ***P* < 0.05.

applied. As demonstrated, the AMPK inhibitor Compound C ("CC") or AMPKα1 shRNAs dramatically inhibited C13-mediated GES-1 cell protection against *H. pylori* (Fig. 3C–D). In addition, Compound C alleviated C13-mediated anti-apoptosis activity in *H. pylori*-infected primary GECs (Fig. 3E). Based on these information, we suggest that C13 enhances *H. pylori*-induced pro-survival AMPK activation to inhibit GEC apoptosis. To exclude the potential involvement of AMPKα2 in C13-induced activity, shRNA method was again applied. Western blot results in Fig. 3F showed that AMPKα2 expression was dramatically inhibited by targeted shRNA in stable GEC-1 cells. AMPKα1 was intact in the same cells (Fig. 3F). C13-mediated apoptosis inhibition against *H. pylori* was not affected by AMPKα2 silencing (Fig. 3G and H). These results suggest that AMPKα2 is likely not required for C13-mediated activity in GECs.

3.4. C13 inhibits H. pylori-induced ROS production in GECs

Studies have shown that ROS production is increased in GECs with *H. pylori* infections, which is associated with GEC apoptosis and gastric carcinogenesis [9]. AMPK activation has the capability of inhibiting oxidative stress [14]. We thus tested the role of C13 on *H. pylori*-induced ROS production. ROS was tested by H2DCFDA FACS assay as described. Results demonstrated that ROS level was increased after *H. pylori* infection in GES-1 cells (Fig. 4A). Notably, C13 co-treatment dose-dependently inhibited *H. pylori*-induced ROS production (Fig. 4A). HO-1 is an important anti-oxidant gene [22]. We discovered that mRNA expression of HO-1 was increased by C13 in GES-1 cells, and the effect was again dose-dependent (Fig. 4B). Significantly, AMPK inhibition by Compound C ("CC"), or by AMPKα1 shRNAs, prevented C13-induced HO-1 expression (Fig. 4C). As expected, Compound C blocked C13-induced AMPK activation in GES-1 cells (Data not shown). On the other hand, A769662, a well-established AMPK activator, induced HO-1 expression in GES-1 cells (Fig. 4B). Importantly, C13-mediated anti-oxidant activity against *H. pylori* was alleviated by Compound C (Fig. 4D), or by the specific HO-1 inhibitors ZnPP (Fig. 4D) and SnPP (Data not shown). As a consequence, treatment with the two HO-1 inhibitors (ZnPP and SnPP) alleviated C13-induced GEC cyto-protective effect against *H. pylori* (Fig. 4E). In primary human GECs, C13 similarly induced HO-1 mRNA expression (Fig. 4F) whiling inhibiting ROS production (Fig. 4G), such effects by C13 were almost blocked by the AMPK inhibitor Compound C (Fig. 4G and H). Together, these results indicate that C13 inhibits *H. pylori*-induced ROS production through activating AMPK–HO–1 signaling.

4. Discussions

H. pylori infection remains one main risk factor in the development of chronic gastritis, peptic ulceration, and gastric carcinoma [1,23,24]. Imbalances between cell apoptosis and proliferation may lead to peptic ulcer and gastric carcinogenesis [24]. Severe *H. pylori* infection is known to induce GEC apoptosis [3–5]. Our previous study demonstrated that *H. Pylori* by itself activated AMPK in GEC-1 cells and primary GECs, which served as a negative regulating signaling to protect GECs from apoptosis [17]. Inhibition of AMPK by Compound C, or by AMPKα1 shRNA, exacerbated *H. Pylori*-induced GEC apoptosis [17]. Here, we showed that C13, the α1-selective small molecule activator of AMPK, potently strengthened *H. Pylori*-induced AMPK activation. As a consequence, ROS production and GEC apoptosis by *H. Pylori* were remarkably

inhibited by C13 co-administration. Pharmacological inhibition or shRNA-mediated silence of AMPKα1 reduced the anti-oxidant and cyto-protective effects of C13 in GECs. Molecularly, we found that C13-activated AMPK induced HO-1 expression to exert its activities in GECs.

AMPK is composed of a catalytic α subunit as well as regulatory β and γ subunits [25,26]. Multiple genes encoding isoforms (α1, α2; β1, β2; γ1, γ2, γ3) as well as transcriptional variants exist for each of the subunits, generating 12 or more distinct heterotrimeric complexes [25,26]. At the cellular level, AMPK plays a role as an energy sensor and regulator. Under conditions of energy depletion, AMPK inhibits ATP-consuming pathways whiling stimulating ATP-generating processes to restore overall cellular energy homeostasis [25,26]. AMPK activation is also important for cellular survival when cells are under stress conditions [14,27,28]. There has been no direct AMPK activators entering clinical trials for treatment of metabolic disorders and possible other diseases [18]. Several small molecules have been reported to directly activate AMPK. The underlying mechanisms of actions of these AMPK activators are, however, mostly not well-elaborated. One except is A769662, which stimulates β1-containing complexes [29]. Recent research efforts have identified a nucleotide mimetic compound 2 (C2) [30], and its cell-permeable pro-drug C13 [18], as novel and highly potent AMPK activators. C13 is characterized as an α1-selective small molecule activator of AMPK [18]. C13 was shown to potently inhibit lipid synthesis in hepatocytes from wild-type and was largely ineffective in AMPKα1-knockout hepatocytes [18]. In the current study, we also found that C13 activates AMPK and inhibit *H. pylori*-induced GEC apoptosis through interacting with AMPKα1. ShRNA-mediated knockdown of AMPKα1 almost abolished C13-mediated activities in GECs.

ROS play major roles in regulating *H. pylori*-associated gastric inflammation and carcinogenesis [9]. Although neutrophils are the main source cells responsible for ROS production in the *H. pylori*-infected stomach, *H. pylori* by itself could also produce ROS [9]. As a matter of fact, recent studies have demonstrated that *H. pylori* could increase ROS production in GECs, which contributes to gastric carcinogenesis through affecting GEC signaling transductions [9]. In addition, excessive ROS in *H. pylori*-infected GECs damage DNAs, a step that is associated with GEC transformation [9]. In this study, we found that C13 dramatically inhibited *H. pylori*-induced ROS production in both transformed and primary GECs. AMPK activation was required for C13-mediated ROS scavenging ability through induction of anti-oxidant gene HO-1 in GECs. Inhibiting or silencing AMPK as well as the HO-1 inhibitors abolished C13-induced anti-oxidant ability in *H. pylori*-infected GECs. In summary, the results of this study demonstrate that C13 protects GECs from *H. pylori*-induced oxidative stress and apoptosis through activating AMPK–HO–1 signaling.

Conflict of interests

No conflict of interests were stated by authors.

Acknowledgments

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References

[1] R.M. Peek Jr., M.J. Blaser, *Helicobacter pylori* and gastrointestinal tract adenocarcinomas, Nat. Rev. Cancer 2 (2002) 28–37.
[2] N. Uemura, S. Okamoto, S. Yamamoto, N. Matsumura, S. Yamaguchi, M. Yamakido, K. Taniyama, N. Sasaki, R.J. Schlemper, *Helicobacter pylori* infection and the development of gastric cancer, N. Engl. J. Med. 345 (2001) 784–789.
[3] F. Yan, H. Cao, R. Chaturvedi, U. Krishna, S.S. Hobbs, P.J. Dempsey, R.M. Peek Jr., T.L. Cover, M.K. Washington, K.T. Wilson, D.B. Polk, Epidermal growth factor receptor activation protects gastric epithelial cells from *Helicobacter pylori*-induced apoptosis, Gastroenterology 136 (2009) 1297–1307 e1291-1293.
[4] A. Bhattacharyya, R. Chattopadhyay, B.R. Burnette, J.V. Cross, S. Mitra, P.B. Ernst, K.K. Bhakat, S.E. Crowe, Acetylation of apurinic/apyrimidinic endonuclease-1 regulates *Helicobacter pylori*-mediated gastric epithelial cell apoptosis, Gastroenterology 136 (2009) 2258–2269.
[5] J. Wei, D. O'Brien, A. Vilgelm, M.B. Piazuelo, P. Correa, M.K. Washington, W. El-Rifai, R.M. Peek, A. Zaika, Interaction of *Helicobacter pylori* with gastric epithelial cells is mediated by the p53 protein family, Gastroenterology 134 (2008) 1412–1423.
[6] X. Fan, S.E. Crowe, S. Behar, H. Gunasena, G. Ye, H. Haeberle, N. Van Houten, W.K. Gourley, P.B. Ernst, V.E. Reyes, The effect of class II major histocompatibility complex expression on adherence of *Helicobacter pylori* and induction of apoptosis in gastric epithelial cells: a mechanism for T helper cell type 1-mediated damage, J. Exp. Med. 187 (1998) 1659–1669.
[7] J. Rudi, D. Kuck, S. Strand, A. von Herbay, S.M. Mariani, P.H. Krammer, P.R. Galle, W. Stremmel, Involvement of the CD95 (APO-1/Fas) receptor and ligand system in *Helicobacter pylori*-induced gastric epithelial apoptosis, J. Clin. Invest. 102 (1998) 1506–1514.
[8] J.M. Houghton, L.M. Bloch, M. Goldstein, S. Von Hagen, R.M. Korah, In vivo disruption of the fas pathway abrogates gastric growth alterations secondary to Helicobacter infection, J. Infect. Dis. 182 (2000) 856–864.
[9] O. Handa, Y. Naito, T. Yoshikawa, *Helicobacter pylori*: a ROS-inducing bacterial species in the stomach, Inflamm. Res. 59 (2010) 997–1003.
[10] D. Carling, C. Thornton, A. Woods, M.J. Sanders, AMP-activated protein kinase: new regulation, new roles? Biochem. J. 445 (2012) 11–27.
[11] S. Wang, P. Song, M.H. Zou, AMP-activated protein kinase, stress responses and cardiovascular diseases, Clin. Sci. Lond. 122 (2012) 555–573.
[12] I. Kim, Y.Y. He, Targeting the AMP-activated protein kinase for cancer prevention and therapy, Front. Oncol. 3 (2013) 175.
[13] M.M. Mihaylova, R.J. Shaw, The AMPK signalling pathway coordinates cell growth, autophagy and metabolism, Nat. Cell. Biol. 13 (2011) 1016–1023.
[14] S.M. Jeon, N.S. Chandel, N. Hay, AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress, Nature 485 (2012) 661–665.
[15] K. Inoki, H. Ouyang, T. Zhu, C. Lindvall, Y. Wang, X. Zhang, Q. Yang, C. Bennett, Y. Harada, K. Stankunas, C.Y. Wang, X. He, O.A. MacDougald, M. You, B.O. Williams, K.L. Guan, TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth, Cell 126 (2006) 955–968.
[16] K. Inoki, T. Zhu, K.L. Guan, TSC2 mediates cellular energy response to control cell growth and survival, Cell 115 (2003) 577–590.
[17] G. Lv, H. Zhu, F. Zhou, Z. Lin, G. Lin, C. Li, AMP-activated protein kinase activation protects gastric epithelial cells from *Helicobacter pylori*-induced apoptosis, Biochem. Biophys. Res. Commun. 453 (2014) 13–18.
[18] R.W. Hunter, M. Foretz, L. Bultot, M.D. Fullerton, M. Deak, F.A. Ross, S.A. Hawley, N. Shpiro, B. Viollet, D. Barron, B.E. Kemp, G.R. Steinberg, D.G. Hardie, K. Sakamoto, Mechanism of action of compound-13: an alpha1-selective small molecule activator of AMPK, Chem. Biol. 21 (2014) 866–879.
[19] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408.
[20] H. Zhang, Y.Y. Liu, Q. Jiang, K.R. Li, Y.X. Zhao, C. Cao, J. Yao, Salvianolic acid A protects RPE cells against oxidative stress through activation of Nrf2/HO-1 signaling, Free Radic. Biol. Med. 69 (2014) 219–228.
[21] G. Bridge, R. Monteiro, S. Henderson, V. Emuss, D. Lagos, D. Georgopoulou, R. Patient, C. Boshoff, The microRNA-30 family targets DLL4 to modulate endothelial cell behavior during angiogenesis, Blood 120 (2012) 5063–5072.
[22] R. Gozzelino, V. Jeney, M.P. Soares, Mechanisms of cell protection by heme oxygenase-1, Annu. Rev. Pharmacol. Toxicol. 50 (2010) 323–354.
[23] E. Garza-Gonzalez, G.I. Perez-Perez, H.J. Maldonado-Garza, F.J. Bosques-Padilla, A review of *Helicobacter pylori* diagnosis, treatment, and methods to detect eradication, World J. Gastroenterol. 20 (2014) 1438–1449.
[24] F. Wang, W. Meng, B. Wang, L. Qiao, *Helicobacter pylori*-induced gastric inflammation and gastric cancer, Cancer Lett. 345 (2014) 196–202.
[25] D.G. Hardie, F.A. Ross, S.A. Hawley, AMPK: a nutrient and energy sensor that maintains energy homeostasis, Nat. Rev. Mol. Cell. Biol. 13 (2012) 251–262.
[26] D.B. Shackelford, R.J. Shaw, The LKB1-AMPK pathway: metabolism and growth control in tumour suppression, Nat. Rev. Cancer 9 (2009) 563–575.
[27] C. She, L.Q. Zhu, Y.F. Zhen, X.D. Wang, Q.R. Dong, Activation of AMPK protects against hydrogen peroxide-induced osteoblast apoptosis through autophagy induction and NADPH maintenance: new implications for osteonecrosis treatment? Cell. Signal 26 (2014) 1–8.

[28] Y. Zhu, J. Zhou, R. Ao, B. Yu, A-769662 protects osteoblasts from hydrogen dioxide-induced apoptosis through activating of AMP-activated protein kinase (AMPK), Int. J. Mol. Sci. 15 (2014) 11190–11203.
[29] S. Ducommun, R.J. Ford, L. Bultot, M. Deak, L. Bertrand, B.E. Kemp, G.R. Steinberg, K. Sakamoto, Enhanced activation of cellular AMPK by dual-small molecule treatment: AICAR and A769662, Am. J. Physiol. Endocrinol. Metab. 306 (2014) E688–E696.
[30] J.E. Gomez-Galeno, Q. Dang, T.H. Nguyen, S.H. Boyer, M.P. Grote, Z. Sun, M. Chen, W.A. Craigo, P.D. van Poelje, D.A. MacKenna, E.E. Cable, P.A. Rolzin, P.D. Finn, B. Chi, D.L. Linemeyer, S.J. Hecker, M.D. Erion, A Potent and Selective AMPK Activator That Inhibits de Novo Lipogenesis, ACS Med. Chem. Lett. 1 (2010) 478–482.