



## Review article

## AMP-activated protein kinase: An emerging target for ginseng

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## ABSTRACT

The adenosine monophosphate (AMP)-activated protein kinase (AMPK) is a key sensor of cellular energy. Once activated, it switches on catabolic pathways generating adenosine triphosphate (ATP), while switching off biosynthetic pathways consuming ATP. Pharmacological activation of AMPK by metformin holds a therapeutic potential to reverse metabolic abnormalities such as type 2 diabetes and nonalcoholic fatty liver disease. In addition, altered metabolism of tumor cells is widely recognized and AMPK is a potential target for cancer prevention and/or treatment. *Panax ginseng* is known to be useful for treatment and/or prevention of cancer and metabolic diseases including diabetes, hyperlipidemia, and obesity. In this review, we discuss the ginseng extracts and ginsenosides that activate AMPK, we clarify the various mechanisms by which they achieve this, and we discuss the evidence that shows that ginseng or ginsenosides might be useful in the treatment and/or prevention of metabolic diseases and cancer.

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## 1. Introduction

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is a somewhat old kinase because its activity was first documented in 1973 as a negative regulator of acetyl-coenzyme A (CoA) carboxylase (ACC) and 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR) in the biosynthesis of fatty acids and cholesterol, respectively [1,2]. AMPK is a highly preserved sensor of cellular energy status, and appears to exist in essentially all eukaryotes as heterotrimeric complexes composed of a catalytic  $\alpha$  subunit and regulatory  $\beta$  and  $\gamma$  subunits. The  $\alpha$  subunit contains the kinase domain, with the conserved threonine residue that is the target for upstream kinases [liver kinase B1 (LKB1) and  $\text{Ca}^{2+}$ -activated calmodulin-dependent kinase kinases (CaMKKs)] located within the activation loop. Phosphorylation at Thr172 is required for kinase activity and function in all species from yeast to man, and with the human kinase, causes >100-fold activation [3]. In mammals, all three subunits have multiple isoforms encoded by distinct genes ( $\alpha 1, \alpha 2; \beta 1, \beta 2; \gamma 1, \gamma 2, \gamma 3$ ), which assemble to form up to 12 heterotrimeric combinations [4]. The functions of the different subunit isoforms remain unclear, although there is tissue-specific

expression of some isoforms, and there is evidence that different isoforms may target complexes to specific subcellular locations. Because the energy status of the cell is a crucial factor in all aspects of cell function, it is not surprising that AMPK has umpteen downstream targets whose phosphorylation mediates dramatic changes in cell metabolism, cell growth, and other functions.

Obesity and the metabolic syndrome represent a major health problem in both Western and developing countries. Considering the role of AMPK in regulating energy balance at both the cellular and whole-body levels, this kinase occupies a pivotal position in studies regarding obesity, diabetes, and the metabolic syndrome [5]. By direct phosphorylation of metabolic enzymes and transcription factors, AMPK switches on catabolic pathways, such as the uptake of glucose and fatty acids, and their metabolism by mitochondrial oxidation and glycolysis. In addition, AMPK switches off anabolic pathways, such as the synthesis of glucose, glycogen, and lipids in the liver. By promoting muscle glucose uptake and metabolism and by inhibiting hepatic gluconeogenesis, AMPK activation can explain the antidiabetic action of metformin. Type 2 diabetes is primarily caused by insulin resistance, which is strongly associated with excess triglyceride storage in liver and muscle. By switching

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off the synthesis of fatty acids and triglycerides and enhancing fat oxidation, AMPK activation might also explain the insulin-sensitizing action of metformin.

The uncontrolled proliferation of cancer cells is supported by a corresponding adjustment of energy metabolism. Nowadays, altered metabolism of tumor cells is widely recognized as an emerging hallmark and a potential drug target in cancer cells. Protein synthesis is the best-characterized process regulated by the mammalian target of rapamycin complex 1 (mTORC1). mTORC1 plays a key role in translational control by phosphorylating lots of translation regulators, including S6 kinase 1 (S6K1) [6]. The synthesis of fatty acids, triglycerides, cholesterol, RNA, and proteins are all upregulated in tumor cells. Notably, because protein synthesis requires a myriad of cellular energy, AMPK activation induced by metabolic stress significantly inhibits protein synthesis, resulting in AMPK–mTORC1 crosstalk: AMPK attenuates mTORC1 signaling through phosphorylation and activation of tuberous sclerosis 2 [7], a negative regulator of mTORC1. AMPK also directly phosphorylates Raptor, which induces 14-3-3 binding to raptor and repression of mTORC1 activity [8]. Other findings that AMPK caused the inhibition of progress through the cell cycle [9], and that the mechanism of AMPK activation required the presence of the tumor suppressor LKB1 [10–12] also gave us the idea that AMPK activators might be beneficial in the prevention and/or treatment of cancer. AMPK activation switches off all of these pathways and would therefore be expected to exert an antitumor effect, reinforced by its ability to cause cell-cycle arrest. These effects of AMPK might explain the tumor suppressor effects of the upstream kinase LKB1 [13], as well as findings that metformin usage reduces the risk of cancer in diabetics [14] and that metformin and other AMPK activators (phenformin, A-769662) delay the onset of tumorigenesis in a mouse model [15].

Over recent years, a plethora of naturally occurring compounds including ginseng and ginsenosides have been reported to activate AMPK in intact cells. These natural products include resveratrol from grapes [16], epigallocatechin-3-gallate (EGCG) from green tea and capsaicin from chili peppers [17], curcumin from turmeric [18], as well as four compounds derived from traditional Chinese medicine, berberine from Chinese Goldthread [19], hispidulin from Snow Lotus [20], licochalcone A from *Glycyrrhiza* and *Brassica rapa* [21], and betulinic acid from *Betula* [22]. Ginseng is one of the most popular and bestselling herbal medicines worldwide. Ginseng has been used as a medicine and/or as a nutraceutical by healthy and ill individuals all around the world. Many clinical and animal studies on ginseng have been performed to characterize its therapeutic properties, which include improving physical performance [23,24] and sexual function [25,26], treating cancer [27,28], diabetes [29–31], and hypertension [32,33]. In this article, we review the mechanisms by which AMPK is activated by ginseng extracts or ginsenosides, well-known active components found in ginseng. Ginseng was used for preventing and/or treating metabolic disorders and cancer prior to when it was realized that ginseng and ginsenosides seem to be AMPK activators.

## 2. Pharmacological activities of ginseng as an AMPK activator

AMPK activators derived from medicinal plants have disparate chemical structures and it was difficult to see how they activate AMPK. However, it has now been shown that some inhibit mitochondrial function, either inhibiting the respiratory chain (berberine and licochalcone A) or the adenosine triphosphate (ATP) synthase (EGCG and resveratrol), or acting as an uncoupler (curcumin). Consistent with the idea that this is how they activate AMPK, berberine and resveratrol increased the AMP:ATP ratio in cultured cells and failed to activate AMPK in cells expressing the

AMP/ADP-insensitive R531G [34]. Why do so many plants produce compounds that are mitochondrial inhibitors and hence AMPK activators? Respiratory chain and ATP synthase might have potential binding sites for xenobiotic compounds, and the production of mitochondrial poisons might be a suitable mechanism for plants to deter infection by pathogens. To date, 31 English language articles were published according to a search of the PubMed database using keywords “ginseng”, “ginsenoside”, and “AMPK”. Among them, 19 articles are related to metabolic diseases, six articles are related to cancer, and six articles are related to other pharmacological activities, including two review articles.

### 2.1. Effects on metabolic diseases

Beneficial effects of ginseng and its active ingredients on metabolic disorders have been known from many clinical and animal studies. Table 1 summarizes the effects of ginseng associated with AMPK activation in animal and cell studies. AMPK phosphorylates serine residues surrounded by a well-defined recognition motif [8,35]. Fig. 1 shows targets involved in the acute and chronic regulation of metabolism. Ginseng or ginsenosides can work on one specific target and pathway or more than one target, or even other targets not shown in Fig. 1, including glycolysis, lipolysis, glycogen synthesis, protein synthesis, forkhead box transcription factor class O1/3a (*FOXO1/3a*) target genes, genes involved in oxidative stress resistance, cytochrome P450 drug metabolism genes, and amplitude and period of expression of circadian genes.

- (1) AMPK activates glucose transporter 4 (GLUT4)-mediated glucose uptake in muscle via phosphorylation of TBC1 domain family member 1 (TBC1D1) [36]. Lee et al [37] demonstrated that higher expression levels of GLUT4 and its transcription factor (myocyte enhancer factor 2, MEF-2) were observed in the gastrocnemius muscle of Korean red ginseng (KRG)-treated Otsuka Long-Evans Tokushima Fatty (OLETF) rats compared with untreated rats.
- (2) AMPK activates fatty acid uptake via translocation of the transporter CD36 to the plasma membrane [38]. Kim et al [39] showed that compound K (CK) increased gene expressions of peroxisome proliferator activated receptor  $\alpha$  (PPAR $\alpha$ ) and CD36, a transcriptional regulator for lipid catabolism and uptake in human hepatoma cells.
- (3) AMPK activates fatty acid oxidation by phosphorylating and inactivating the mitochondria-associated isoform of ACC2, thus lowering malonyl-CoA, an inhibitor of fatty acid uptake into mitochondria via the carnitine palmitoyltransferase system [40]. Shen et al [41] demonstrated that Rb1 reduced fatty liver in obese rats, and this effect was primarily due to increased fatty acid oxidation via activation of the AMPK signaling pathway.
- (4) AMPK inhibits fatty acid synthesis by directly phosphorylating and inactivating the cytosolic isoform of ACC1 [42].
- (5) AMPK inhibits triglyceride and phospholipid synthesis by causing inactivation of the first enzyme involved in their synthesis, glycerol-3-phosphate acyl transferase (GPAT) [43]. Yuan et al [44] demonstrated that CK has a beneficial effect on lipid metabolism via activation of AMPK in the liver of C57BL/ksj db/db mice. CK (also known as IH-901) significantly reduced the expressions of sterol response element binding protein 1 (SREBP1) and its target genes such as fatty acid synthase (*FAS*), stearoyl-CoA desaturase 1 (*SCD1*), and *GPAT* in the liver of mice.
- (6) AMPK inhibits cholesterol synthesis by direct phosphorylation and inactivation of HMGCR [45]. Lee et al [46] showed that ginsenoside Rg3 reduces lipid accumulation in HepG2 cells. Rg3 decreased mRNA expression of SREBP2, a transcriptional regulator of genes involved in cholesterol metabolism, and expression of *HMGCR*, which catalyzes a rate-limiting step in

**Table 1**  
Effects of Ginseng on Metabolic Diseases in Relation to AMPK Activation

| Material    | Cell line/animal                                   | Dose/duration                   | Effects and molecular mechanism   | Refs |
|-------------|--|---------------------------------|---|------|
| Rb1         | HFD-fed Long-Evans rats<br>Rat primary hepatocytes | 10 mg/kg, i.p. for 4 weeks      | Decreased hepatic fat accumulation<br>Enhanced fatty acid oxidation via increase in CPT1 activity<br>Increased AMP/ATP ratio                          | [41] |
| Rb2         | H4IIE cells  | 0.01–1 $\mu$ M                  | Inhibited gluconeogenesis via induction of <i>SHP</i> gene expression and suppression of ROS–JNK pathway in palmitate-induced insulin resistance      | [56] |
| Rc          | C2C12 myotubes                                     | 50–200 $\mu$ M                  | Induced glucose uptake and p38 MAPK phosphorylation<br>AMPK and p38 activation was mediated by ROS production   | [57] |
|             | HepG2 cells  | 0.1–10 $\mu$ M                  | Decreased hepatic triglyceride and cholesterol levels<br>Inhibited expression of SREBP-2 and HMGR   | [46] |
| Rg3         | 3T3-L1 adipocytes                                  | 20–80 $\mu$ M                   | Inhibited adipocyte differentiation by activation of AMPK and inhibition of PPAR- $\gamma$  | [58] |
|             | C2C12 myotubes                                     | 10–100 $\mu$ M                  | 20(S)-Rg3 showed higher pharmacological effects in insulin secretion and AMPK activation than 20(R)-Rg3   | [59] |
|             | 3T3-L1 adipocytes                                  | 0.001–0.1 $\mu$ M               | Enhanced glucose uptake and stimulated GLUT4 translocation by activation of AMPK and PI3K pathway<br>Inhibited TG accumulation                        | [60] |
| CK (IH-901) | C57BL/KsJ db mice                                  | 10–25 mg/kg, p.o. for 6 weeks   | Plasma glucose decreased by 20.7% at 25 mg/kg<br>Plasma insulin increased by 3.4 times in 25 mg/kg-treated mice                                       | [44] |
|             | C2C12 myotubes                                     | 5–20 $\mu$ M                    | Stimulated glucose uptake and overexpression of GLUT4 via activation of AMPK and PI3K–Akt pathway<br>Inhibited TG accumulation                        | [39] |
|             | HepG2 cells  | 5–20 $\mu$ M                    | Inhibited lipogenesis by modulating LKB1–AMPK–SREBP1 signaling pathway, and stimulated lipolysis via upregulations of PPAR- $\alpha$ and CD36         | [50] |
| Re          | HepG2 cells  | 5–20 $\mu$ M                    | Inhibited hepatic glucose production and lipogenesis via activation of AMPK signaling pathway   | [50] |
|             | HFD-fed C57BL/6J mice                              | 5–20 mg/kg, p.o. for 3 weeks    | Lowered blood glucose and TG levels by 18.9% and 29.5% in 20 mg/kg of Re-treated mice   | [60] |
|             | 3T3-L1 adipocytes                                  | 0.001–0.1 $\mu$ M               | Enhanced glucose uptake by inducing mRNA and protein expression of GLUT4  | [60] |
| Rg1         | C2C12 myotubes                                     | 10–40 $\mu$ M                   | Enhanced TG accumulation<br>Improved insulin resistance<br>Enhanced glucose uptake by overexpression of GLUT4 via activation of AMPK                  | [61] |
|             | HepG2 cells  | 10–40 $\mu$ M                   | Inhibited hepatic glucose production by phosphorylation of LKB1, AMPK, and FoxO1<br>PEPCK and G6Pase activities were decreased                        | [55] |
| Rg2         | HepG2 cells  | 5–20 $\mu$ M                    | Inhibited hepatic glucose production by phosphorylation of GSK $\beta$ and induction in <i>SHP</i> gene expression was mediated by<br>AMPK activation | [48] |
| KRG         | Otsuka Long-Evans<br>Tokushima Fatty rats          | 200 mg/kg, p.o. for 40 weeks    | Improved insulin sensitivity<br>Promoted fatty acid oxidation and enhanced mitochondrial biogenesis and glucose utilization by activation of AMPK     | [37] |
| Ginsam      | Otsuka Long-Evans<br>Tokushima Fatty rats          | 300–500 mg/kg, p.o. for 8 weeks | Plasma insulin levels were lowered, and this effect was related to overexpression of GLUT4 by activation of AMPK and PPAR- $\gamma$                   | [62] |

CK, compound K; CPT1, carnitine palmitoyltransferase-1; FoxO1, forkhead box class O1; G6Pase, glucose-6-phosphatase; GLUT4, glucose transporter 4; GSK3 $\beta$ , glycogen synthase kinase 3 $\beta$ ; HFD, high fat diet; HMGR, 3-hydroxy-3-methyl glutaryl coenzyme A reductase; i.p., intraperitoneal administration; JNK, c-Jun NH2-terminal kinase; KRG, Korean red ginseng; LKB1, liver kinase B1; MAPK, mitogen-activated protein kinase; PEPCK, phosphoenolpyruvate carboxykinase; PI3K, phosphatidylinositol 3-kinase; p.o., oral administration; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; SHP, orphan nuclear receptor small heterodimer partner; SREBP, sterol regulatory element binding protein; TG, triglyceride.

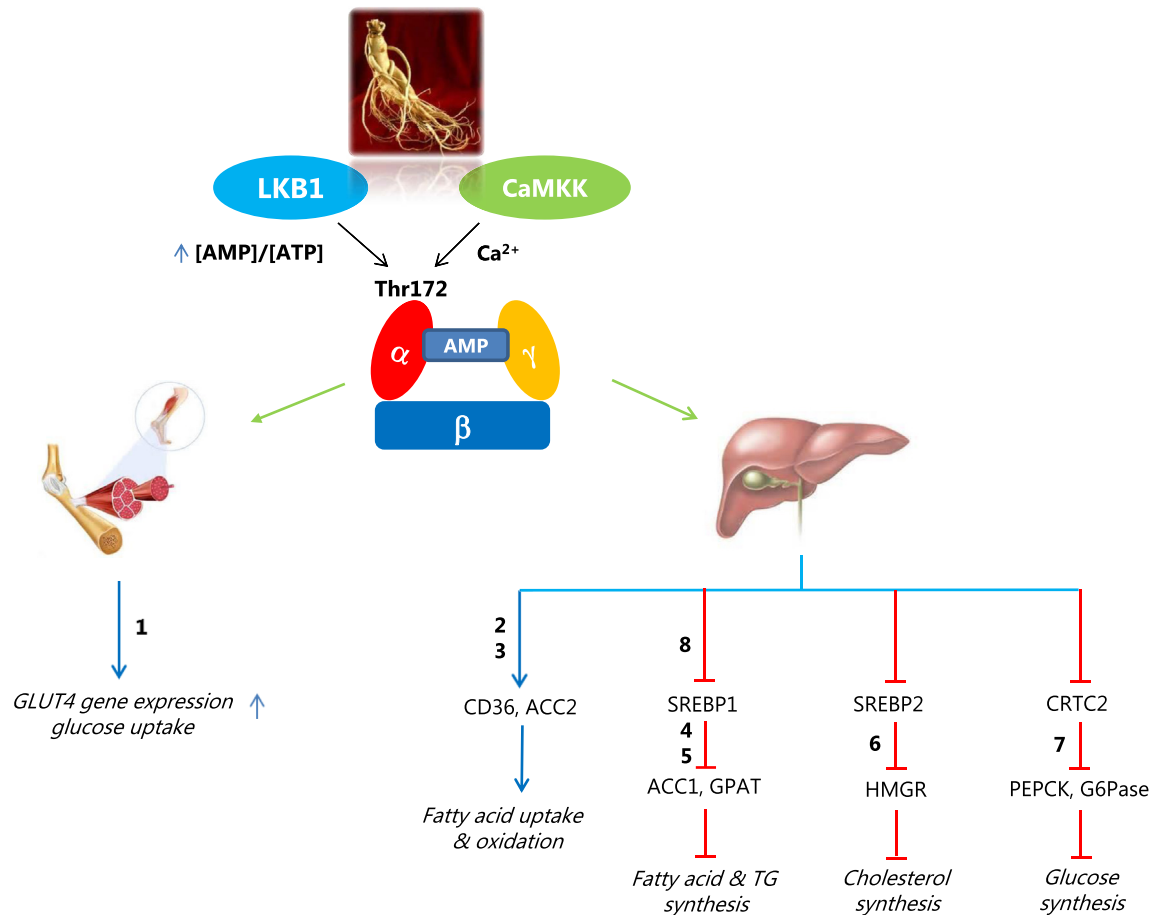
cholesterol synthesis, was also suppressed in a time-dependent manner.

(7) AMPK phosphorylates cyclic AMP response element binding protein (CREB)-regulated transcription coactivator-2 (CRTC2), causing it to bind 14-3-3 proteins, thus retaining it in the cytoplasm and inhibiting activation of genes encoding gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase (*PEPCK*) and glucose-6-phosphatase (*G6Pase*) in the liver [47]. Yuan et al [48] revealed that ginsenoside Rg2 inhibits hepatic glucose production through activation of the AMPK signaling pathway. Although phosphorylation of CRTC2 by Rg2 was not shown in this article, Rg2 reduced phosphorylation of CREB causing interruption of the formation of the CREB–CRTC2 complex, which resulted in suppression of gene expression of gluconeogenic enzymes such as *PEPCK* and *G6Pase*.

(8) AMPK phosphorylates SREBP1, preventing its proteolytic processing and translocation into the nucleus and thus inhibiting transcription of lipogenic genes, including those encoding ACC1 and FAS [49]. Our group reported that CK and Re attenuate hepatic lipid accumulation in HepG2 cells [39,50]. CK and Re attenuated the expression of *SREBP1*, central to the intracellular surveillance of lipid catabolism and *de novo* biogenesis, in time- and dose-dependent manners. Genes for SCD1 and FAS, well-known target molecules of SREBP1, were also suppressed.

## 2.2. Effects on cancer

Beneficial effects of ginseng or ginsenosides on cancer associated with the AMPK signaling pathway were reported since 2009,



**Fig. 1.** Acute and chronic metabolic effects of adenosine monophosphate (AMP)-activated protein kinase (AMPK) activation. See text for numbering and key to acronyms. Blue arrows indicate activation, red lines with a bar at the end indicate inhibition. Suppression of hepatic glucose production and lipid accumulation by ginsenosides were mainly mediated by liver kinase B1 (LKB1)–AMPK signaling pathways. ACC, Acetyl coenzyme A carboxylase; ATP, adenosine triphosphate; CaMKK, calmodulin-dependent kinase kinase; CRTC, regulated transcription coactivator; GLUT4, glucose transporter 4; G6Pase, glucose-6-phosphatase; GPAT, glutamine phosphoribosylpyrophosphate amidotransferase; HMGR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; PEPCK, phosphoenolpyruvate carboxykinase; SREBP, sterol response element binding protein; TG, triglyceride.

and there are six articles published up to the present time. Recently, our group reported that CK and Rg3 induce apoptosis via the CaMKK–AMPK signaling pathway in HT-29 colon cancer cells, and these activities were confirmed using either compound C (a chemical inhibitor of AMPK) or small interfering RNA (siRNA) for AMPK or STO-609 (a chemical inhibitor of CaMKK) [51,52]. Kim et al [53] also reported that CK inhibits cell growth, induces apoptosis via generation of reactive oxygen species, as well as decreasing cyclooxygenase-2 expression and prostaglandin E2 levels. These effects were induced via an AMPK-dependent pathway and were abrogated by a specific AMPK inhibitor, compound C [53]. More recently, Hwang et al [54] reported that 20-O- $\beta$ -D-glucopyranosyl-20(S)-protopanaxadiol (20-GPPD), a metabolite of ginseng saponin, causes apoptosis of colon cancer cells through the induction of cytoplasmic  $Ca^{2+}$ . 20-GPPD decreased cell viability, increased annexin V-positive early apoptosis, and induced sub-G1 accumulation and nuclear condensation of CT-26 murine colon cancer cells. Although 20-GPPD-induced activation of AMPK played a key role in the apoptotic death of CT-26 cells, LKB1, a well-known upstream kinase of AMPK, was not involved in this activation [54].

Although many studies support the tumor-suppressive role of AMPK, some evidence suggests that the metabolic function of AMPK might be overridden by oncogenic signals so that tumor cells use AMPK activation as a survival strategy to gain growth. During certain stages of tumor development, AMPK might act as protective

machinery against metabolic stress such as nutrient deprivation and hypoxia. Thus, investigation to define at which stage of cancer progression might represent a more relevant strategy to employ AMPK activation for cancer treatment is clearly warranted.

### 3. Perspectives

AMPK is a critical metabolic sensor that finely regulates the energy homeostasis of cells. Therefore, it has been suggested as a potential target for metabolic disorders and cancer. A plethora of chemical agents reported to activate AMPK exist, most notably metformin and 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR). Most of these chemicals, except A-769662, known to be a direct AMPK activator developed in 2005 by Abbott Laboratories, Abbott Park, Illinois, USA, activate AMPK indirectly with some other effects. At this time, we do not know exactly how ginseng or ginsenosides activate AMPK although LKB1 [39,48,50,55] or the calcium-dependent pathway involving phosphorylation of AMPK by CAMKK would be suggested. As alternative or additional explanations, mechanisms involving either an increase in the AMP:ATP ratio [41], inhibition of mitochondrial ATP synthesis, or the SIRT1-dependent pathway via increase in nicotinamide adenine dinucleotide ( $NAD^+$ ) levels should be tested to elucidate further how ginseng or ginsenosides activate AMPK. Despite recent advances in the mechanistic understanding of AMPK activation by ginseng or ginsenosides,



several key questions still remain. Is there a positive correlation between antimetabolic or anticancer activities of ginseng (and ginsenosides) and the AMPK signaling pathway as a primary target? If yes, how do ginseng or ginsenosides activate AMPK? Do they activate AMPK directly or indirectly? What are the therapeutic and toxicological consequences of AMPK activation? The AMPK field of research is now well developed and should provide new and exciting novelties regarding the application of AMPK in preventive and clinical medicine. With the concerted research efforts of many laboratories, these challenges may be addressed soon.

### Conflicts of interest

All authors declare no conflicts of interest.

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