Pharmacological Effects of 1′-Acetoxychavicol Acetate, a Major Constituent in the Rhizomes of *Alpinia galanga* and *Alpinia conchigera*

Akiko Kojima-Yuasa, Isao Matsui-Yuasa

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Akiko Kojima-Yuasa and Isao Matsui-Yuasa

Department of Food and Human Health Sciences, Graduate School of Human Life Science, Osaka City University, Osaka 558-8585, Japan

Correspondence to: Dr. Akiko Kojima-Yuasa, Department of Food and Human Health Sciences, Graduate School of Human Life Science, Osaka City University, Osaka 558-8585, Japan.

E-mail: kojima@life.osaka-cu.ac.jp

Tel: (+80) 6-6605-2865, Fax: (+80) 6-6605-2810

ABSTRACT

1'-Acetoxychavicol acetate (ACA) is found in the rhizomes or seeds of *Alpinia galanga* and *Alpinia conchigera*, which are used as traditional spices in cooking and traditional medicines in Southeast Asia. ACA possesses numerous medicinal properties. Those include anticancer, anti-obesity, antiallergy, antimicrobial, antidiabetic, gastroprotective, and anti-inflammatory activities. ACA is also observed to exhibit antidementia activity. Recent studies have demonstrated that combining ACA with other substances results in synergistic anticancer effects. The structural factors which regulates the activity of ACA include: (1) the acetyl group at position 1', (2) the acetyl group at position 4, and (3) the unsaturated double bond between positions 2' and 3'. ACA induces the activation of AMPK, which regulates the signal transduction pathways, and has an important role for the prevention of diseases, including cancer, obesity, hyperlipidemia, diabetes, and neurodegenerative disorders. Such findings suggest that AMPK has a central role in different pharmacological functions of ACA, and ACA is useful for the prevention of life-threatening diseases. However, more studies should be performed to evaluate the clinical effects of ACA and to better understand its potential.

Keywords: 1'-Acetoxychavicol acetate, *Alpinia galanga*, *Alpinia conchigera*, Diverse pharmacological effects, Synergistic Anticancer Treatment Effects, Structure-Activity Relationships, AMPK

INTRODUCTION

1'-Acetoxychavicol acetate (ACA; Figure 1) is found in the rhizomes or seeds of *Alpinia galanga* and *Alpinia conchigera*, which are plants in the ginger family. *Alpinia galanga* and *Alpinia conchigera* are commonly called "greater galangal" and "lesser Alpinia", respectively. These are used as traditional spices in cooking, especially the former is used in Thai and Indonesian cuisines, while the latter grows in eastern Bengal, southern peninsular Malaysia, and Sumatra. These rhizomes have often been used as traditional medicines for gastrointestinal disorders in Thailand and fungal infections in Malaysia.

To date, ACA, the main component of the crude ethanolic extract of rhizomes, has exhibited diverse medicinal properties like anticancer effects, gastroprotective effects, xenobiotic protection, antiallergic activity, antimicrobial activity, and antidementia effects. In this review, we aimed to summarize the diverse bioactivities of ACA, which are presented in Tables 1 and 2, the efficacy of combined treatment with ACA and other substances (Table 3), and discuss the relationships between structure and pharmacological activities of ACA and the involvement of AMP-activated protein kinase (AMPK) activation in its different pharmacological functions.

Pharmacological effects of ACA

Anticancer effects of ACA

Cancer is the second-leading cause of death in the world. Therefore, numerous efforts have been dedicated to establishing preventive methods and discovering new treatments.

ACA is one such preventive method and has been reported to exhibit potent anticancer effects against various tumor cells, such as colorectal adenocarcinoma cells, cervical cancer cells, oral squamous carcinoma cells, prostate cancer cells, breast cancer cells, glioblastoma cells, myeloma cells, hepatocellular carcinoma cells, and Ehrlich ascites tumor cells.

After examining the antitumor activity of ACA with a convenient short-term *in vitro* assay that

involved tumor promoter-induced Epstein-Barr virus (EBV) activation in 1993, Kondo *et al.* became the first to report that ACA has anticancer effects. Based on their results, the rhizome extract and its main component, ACA, potently inhibited EBV activation induced by a tumor promoter, 12-O-tetradecanoylphorbol-13-acetate [1, 2]. Thereafter, many researchers identified the influences of ACA in the initiation step of carcinogenesis via *in vivo* experiments. Among them, Ohnishi *et al.* found that ACA ingestion during the initiation phase effectively inhibited 4-nitroquinoline 1-oxide-induced oral carcinogenesis in male F344 rats [3]. ACA also exhibited preventive effects on chemical carcinogenesis in the skin. Murakami *et al.* also examined the effect of ACA in a two-stage carcinogenesis experiment in ICR mouse skin and found that topical application of ACA markedly reduced the average number of tumors [4]. Furthermore, Tanaka *et al.* found that dietary ACA effectively suppressed the development of colonic crypt foci induced by azoxymethane in rats when ingested during or after the initiation phase [5]. Kobayashi *et al.* demonstrated that ACA prevents endogenous rat liver carcinogenesis in male Fisher 344 rats by preventing oxidative DNA damage [6]. Feeding ACA in diet at the initiation step inhibited the cholangiocarcinogenesis induced by *N*-nitrosobis(2-oxoprooyl)amine in hamsters [7] and esophageal tumorigenesis induced by *N*-nitrosomethylbenzylamine in male F344 rats [8].

In 2001, Mori *et al.* found that ACA causes apoptosis and suppresses cell proliferation [9]. Many reports exist on the mechanism of apoptosis induced by ACA. In fact, Ito *et al.* indicated that ACA induced apoptosis, and was independently mediated via the mitochondrial oxidative stress pathway and Fas-induced apoptotic pathway in myeloid leukemia [10]. They also reported that ACA suppresses the proliferation of human myeloma cells by inhibiting the translocation of nuclear factor κB (NF-κB) to nuclei, and then induces the apoptosis of myeloma cells [11]. Ichikawa *et al*. reported that ACA enhances apoptosis and inhibits invasion by blocking NF-κB activation using different agents and numerous human cell lines [12]. Batra *et al.* indicated that ACA inhibits skin tumorigenesis in constitutive Stat3 expressed transgenic mice (K5.Stat3C)

by suppressing NF-κB activation [13]. Moreover, Ito *et al.* revealed that ACA upregulates the expression of tumor necrosis factor-related apoptosis-inducing ligand/Apo2 ligand (TRAIL/Apo2L) and TRAIL receptor death receptor 5 in the apoptotic pathway of human myeloma cells [14]. Campbell *et al.* also reported that ACA increases the expression of caspase-3 in human breast carcinoma cells [15].

The efficacy of the antitumor activity of ACA, which is reported as a suppressor of carcinogenesis, on polyamine (putrescine, spermidine and spermine) metabolism was examined in Ehrlich ascites tumor cells (EATCs). Polyamines are necessary for the proliferation and differentiation of cells. In our laboratory, Moffatt *et al.* found that the antitumor effects of ACA were derived from the perturbation of the late limiting enzymes in the polyamine metabolic pathway and the enhancement of caspase-3-like activity [16]. They also found that ACA decreased in phosphorylated retinoblastoma (Rb) protein, and induced nuclear localization of $p27^{kip1}$, followed the accumulation of G1 phase of EATC [17,18]. Furthermore, these events were demonstrated to be cellular thiol-dependent [19-21]. Baradwaj *et al.* confirmed that the mode of Dukes' type B colorectal adenocarcinoma (SW480) cell death with ACA was due to apoptosis and the G0/G1 arrest [22]. In addition, they observed an increase in p21 expression and a concomitant decrease in cyclin D in SW480 cells treated with ACA.

ACA caused the suppression of angiogenesis-mediated prostate tumor growth. Angiogenesis plays an important role in cancer metastasis. By examining the effect of ACA on anti-angiogenesis activity, Pang *et al.* found that ACA inhibited the vascular endothelial growth factor-induced migration, proliferation, adhesion and tubulogenesis of human umbilical vascular endothelial cells [23].

Recently, several researchers have published intriguing findings that ACA suppresses the microRNA (miRNA) expression in several cancer cells. miRNAs are highly conserved small noncoding RNAs that post-transcriptionally regulate gene expression.

miRNAs bind to target sequences in the noncoding regions of target messenger RNAs and

induce messenger RNA degradation or suppress translation [24]. An miRNA is able to regulate many genes; a gene can be targeted by different miRNAs; and each miRNA can regulate human tumorigenesis, cell proliferation, metabolism, or apoptosis [25]. Phush *et al*. examined the combined effects of ACA and cisplatin, a platinum-based anticancer drug, on HPV-positive human cervical carcinoma cell lines, and identified the miRNAs regulated in response to ACA and/or cisplatin. They reported that ACA synergistically enhances the cytotoxic effects of cisplatin by dysregulating specific miRNAs in cervical carcinoma cells. These researchers also emphasized that alterations in miRNA expression affect many important signaling pathways, such as the ERK pathway [26]. Wang *et al*. demonstrated that ACA inhibits the proliferation of HN4, a human head and neck squamous cell carcinoma cell line, and downregulates miRNA-23a expression. They confirmed that phosphatase and tensin homologs deleted on chromosome 10 (PTEN) are the targets of miRNA-23a [27] and proceeded to demonstrate that the downregulation of miRNA-210 conferred sensitivity toward ACA in cervical cancer cells by binding to sequences in the 3' noncoding region of the Smad4 mRNA [28]. Recently, Phuah *et al.* reported that sensitivity to ACA was increased by the suppression of miRNA-629 with reducing cell growth and inducing apoptosis in cervical cancer cells [29]. These results suggest that combining miRNAs and natural compounds like ACA, could become new strategies to treat cervical cancer. These and other reports on anticancer effects are shown in Table 1.

Antiobesity effects of ACA

Obesity is one of the main risk factors for type 2 diabetes, cardiovascular disease, and chronic kidney disease. Several drugs that decrease appetite, fat absorption, and fat oxidation have been developed to treat obesity. However, many have disappeared or been removed from the market due to low efficacy and side effects. Natural compounds cause minimal side effects, consequently they are excellent sources for developing new drugs. Therefore, use of natural

compounds may be a promising anti-obesity strategy[30].

Narukawa *et al.* found that ACA acts as an agonist to activate transient receptor potential cation channel subfamily V, member 1 (TRPA1) [31]. Furthermore, cinnamaldehyde, the TRAP1 agonist, is involved in the reduction of visceral body fat and exhibits anti-obesity effects [32]. These results indicate that ACA may have anti-obesity effects.

By investigating the anti-obesity effect of ACA, Ohnishi *et al.* found that ACA inhibits cellular lipid accumulation by downregulating transcription factors, such as PPARγ and C/EBPα in 3T3-L1. ACA also caused the activation of AMPK. In an *in vivo* animal obesity model, rats fed a high fat diet (HFD) with 0.05% ACA gained significantly less body weight than rats fed an HFD alone. Additionally, the visceral fat mass in rats fed an HFD with 0.05% ACA was lower than that in rats fed HFD alone [33]. Recently, many substances with anti-obesity effects that are regulated by the activation of the AMPK signaling pathway have been reported [34-36]. These findings suggest that AMPK mediates the anti-obesity effect of ACA.

Gastroprotective effects of ACA

In 1976, Mitsui *et al.* found that ACA had strong inhibitory effect against Shay ulcer in rats [37]; Shay ulcer was established through pylorus ligation according to Shay's method.

Owing to the absence of studies on the gastroprotective effect of ACA using other ulcer models, Matsuda *et al.* opted to examine its effect on other ulcer models. Based on their results, ACA significantly protected against ethanol-induced gastric mucosal lesions [38]. Furthermore, ACA prevented 0.6 M HCl-and aspirin-induced gastric mucosal lesions. However, the gastroprotection of ACA was inhibited by pretreatment with indomethacin and N-ethylmaleimide, an SH-blocker. Such findings indicate that the protective effect of ACA may be associated with endogenous prostaglandins and intracellular glutathione (GSH) levels. By performing a further examination of the structure-activity relationship of ACA to elucidate its

gastroprotective effect using various related compounds, these researchers also reported that the 1'-acetoxyl group of ACA is essential for this effect[38].

Free radicals by oxygen and lipid peroxidation are considered to be factors that cause gastrointestinal lesions. Therefore, these results indicate that the GSH levels increased by ACA in gastric mucosa may prevent the increase in free radicals and lipid peroxidation and then prevent lesions caused by different ulcers.

Xenobiotic protective activities of ACA

Xenobiotic metabolism that alters the structure of exogenous or endogenous chemicals, is an important metabolism for maintaining homeostasis and cell conservation. The xenobiotic metabolism pathway consists of three steps: Phase I, Phase II and Phase III. In Phase I, xenobiotics are modified by hydrolysis, oxidation, and reduction and ultimately become the active metabolites. In Phase II, the phase I product is converted to hydrophilic products by transferase enzymes. In Phase III, the final modified product is excreted from the cells via different transporters. The detoxification process is catalyzed by the cytochrome P450 (CYPs) family, the UDP-glucuronosyltransferases (UGT) family, the glutathione S-transferases (GST) family, and the aldehyde dehydrogenase family. In particular, UGT and GST families in Phase II play important roles to determine the rate of xenobiotic metabolism.

Phytochemicals, such as flavonoids, sulforaphane, and curcumin, induce the phase II enzyme activities. When examining the effect of ACA on colon tumorigenesis induced by azoxymethan, Tanaka *et al.* found that dietary ACA inhibited tumorigenesis by suppressing cell growth in the colonic mucosa and inducing phase II detoxification enzymes, GST and NAD(P)H: quinone oxidoreductase (NQO), in the liver and colon [5].

In our research group, Yaku *et al.* revealed that ACA induces GST and NQO, in normal cells and rat intestine epithelial cells (IEC-6). They also found that the induction of phase II enzymes with ACA was caused by the upregulated levels of intracellular nuclear factor, erythroid 2 (NF-

E2)-related factor-2 (NrF2), and cytosolic p21 [39].

Antiallergy effects of ACA

Allergies are hypersensitivity disorders of the immune systems. Matsuda *et al.* found that ACA could suppress the secretion of β-hexosaminidase, a marker of antigen- immunoglobin E-mediated degranulation, in rat basophilic leukemia cells [40]. β-Hexosaminidase accumulates in the secretory granules of mast cells and is concomitantly released with histamine when mast cells are activated immunologically [41]. Matsuda *et al*. also reported that ACA inhibits the release of tumor necrosis factor-α and interleukin-4, which are involved in the late phase of type I allergic reaction [40]. Furthermore, ACA suppressed ear passive cutaneous anaphylactic reactions in mice.

Asthma is an immune-mediated disease that is associated with an imbalance between T helper subsets. This imbalance causes the upregulation of cytokines and then promotes chronic inflammation in the respiratory system.

By examining the effect of ACA in an ovalbumin-induced asthma mouse model, Seo *et al*. found that ACA dose-dependently reduced white blood cell infiltration in the lungs of the model mouse [42]. This increase in white blood cell infiltration is characteristic of asthma.

Antimicrobial effect of ACA

The antimicrobial activity of *Alpinia galanga* extract was previously reported [30]. In Thailand, Phongpaichit *et al.* extracted many medicinal plants with chloroform, methanol, and water and evaluated their antimycobacterial activity against *Mycobacterium tuberculosis* H37 Ra [43]. As a result, they found that the chloroform extract of *Alpinia galanga* was most active. The minimum inhibitory concentration (MIC) was 0.12 μg/mL. This MIC was almost the same as that of isoniazid, an antituberculosis drug (0.1 μg/mL). Furthermore, after isolating ACA from a chloroform extract of *Alpinia galanga* and evaluating its antimycobacterial activity, they found

that ACA has very strong antimycobacterial activity with an MIC of 0.024 μg/mL.

Recently, Warit *et al.* examined the effects of the *S*-enantiomer of ACA (S-ACA), isolated from *A. galangal*, and the synthetic racemic 1'-*R, S*-ACA (rac-ACA) on the cytocidal activity of *Mycobacterium tuberculosis* H37Ra and H37Rv. Based on their findings, S-ACA and rac-ACA had MICs of 0.5 μg/mL and 2.7 μg/mL, respectively. [44] These findings suggest that S-ACA exhibits potent bactericidal activity.

Latha *et al*. reported that ACA has an anti-plasmid activity against multidrug resistant bacteria [45]. Furthermore, ACA showed antibacterial activity against the anaerobic bacterium, *Propionibacterium acnes* [46].

Effect of ACA on proteasome activity

The ubiquitin-proteasome-system, one of the protein degradation systems, is an important pathway for the degradation of misfolded, old-, and short-lived proteins. However, proteasome activity decreases in an age-dependent manner and causes age-related neurodegenerative processes.

Based on the relationship between proteasome activity and ACA in the neurodegenerative process, Yaku *et al.* examined the effect of ACA on proteasome activity and cell protection against neurotoxicity using differentiated PC12 cells. Based on their findings, ACA enhanced proteasome activity during the early stages of ACA treatment in PC12 cells [47]. Furthermore, they reported that the increased proteasome activity was inhibited by H-89, a cAMP-dependent protein kinase inhibitor. ACA also protected against amyloid β-protein fragment induced-neurotoxicity in differentiated PC12 cells. On the other hand, the treatment with MG132, a proteasome inhibitor, reduced the protective effect of ACA. Altogether, these findings demonstrate that ACA induces protease activity by activating the cAMP/PKA signaling pathway in neuronally differentiated PC12 cells.

Antidementia effect of ACA

Alzheimer's disease (AD) is an irreversible progressive neurodegenerative disease with age-dependent and impaired cognitive function and thinking ability. In our laboratory, the effect of ACA on learning and memory with ageing was examined using senescence-accelerated mice prone 8 (SAMP8), a suitable AD model [48]. The learning and memory abilities of SAMP8 fed the 0.02% ACA diet using the Morris water maze test were significantly improved compared to those of SAMP8 mice fed the control diet.

We also examined short-term memory by observing the spontaneous alternations of mice using the Y-maze test. The spontaneous alterations in SAMP8-control mice were significantly decreased compared to the senescence-accelerated resistant/1 (SAMR1) mice. On the other hand, the cerebral tissue in SAMR1 mice was observed to be normal. However, in SAMP8-control mice, neuronal damage in the hippocampal CA1 region was observed. Nonetheless, the hippocampal CA1 region in SAMP8-ACA mice were almost normal, similar to that of SAMR1. The study also showed that ACA increased the serum concentration of β-hydroxybutyric acid, and suggested that this may be involved in the amelioration of cognitive function in SAMP8-ACA mice.

β-Hydroxybutyric acid is presumed to assume roles such as an alternative energy to glucose and an inhibitor of histone deacetylase; however, its uptake into neurons is reduced by aging or an epigenetic action [49]. Further research is thus needed to draw definitive conclusions.

These and other reports on the physiological effects of ACA, except its antitumor activity, are summarized in Table 2.

Effects of combination treatment with ACA and other substances

Many studies have reported that combination treatments with natural products and other substances, such as chemotherapy agents, may be more efficacious than higher or maximal doses of a single agent [50]. Recently, the effects of combination treatment with ACA and other

substances were examined in several experimental systems.

In *et al.* examined the effect of a combination of ACA and cisplatin in oral squamous cell carcinoma cells (SSC) and *in vivo* human oral tumor xenografts using Nu/Nu mice [51]. In SSC, combined treatment with ACA and cisplatin was found to enhance the cytotoxic effects in a synergistic manner. Furthermore, ACA was found to potentiate the effect of cisplatin and thereby caused a reduction in tumor volume.

Phuah *et al.* also investigated the effect of ACA combined with and cisplatin on HPV-positive human cervical carcinoma cells [26]. They found that combined treatment induced cytotoxity dose- and time-dependently and enhanced the expression of 25 miRNAs, including has-miR-138, has-miR-210, and has-miR-744, with predicted gene targets involved in the signaling pathways that regulate apoptosis and cell cycle progression. Such findings suggest that miRNAs have an important role in the the response of anticancer drug to ACA, and synergistically potentiate the effect of cisplatin as a chemosensitizer.

Previously, we investigated the effect of the combination of ACA and sodium butyrate. Moreover, Kato *et al.* evaluated the combined effect of ACA and sodium butyrate on the proliferation of HepG2 human hepatocellular carcinoma cells in our laboratory [52]. In combination treated cells, cell numbers were synergistically decreased via apoptosis; however, this decrease was suppressed in the cells pretreated with catalase, which also caused a significant increase in NADPH oxidase activities and intracellular reactive oxygen species (ROS) levels. Such findings suggest that an increase in intracellular ROS levels relates with cancer cell death. AMPK phosphorylation was also significantly induced in cells treated with the combination; however, this induction was suppressed in the cells pretreated with catalase, suggesting that a increase in ROS is involved in the enhancement of phosphorylation of AMPK. These results indicate that combination with ACA and sodium butyrate synergistically induces apoptosis by increasing intracellular ROS and AMPK phosphorylation.

Yaku *et al.* also examined the effect of the combination of ACA and sodium butyrate on

phase II enzyme activities in intestinal epithelial cells in our laboratory [53]. ACA and sodium butyrate synergistically increased phase II enzyme activities and promoted p53 acetylation. Furthermore, the inhibition of AMPK activity was found to reduce the phase II enzymes. Such findings suggest that the combined treatment of ACA and sodium butyrate synergistically upregulates phase II enzyme activities through activation of AMPK and acetylation of p53.

Arshad *et al*. examined the effect of ACA and recombinant human alpha fetoprotein (rhAFP) on human cancer xenografts in athymic nude (Nu/Nu) mice [54]. According to their findings, mice administered the combined treatment of rhAFP and ACA had a synergistic reduction in tumor volume compared to those administered each agent alone. The combined treatment could not only inhibit the translocation of NF-κB to the nuclei but also decreased the expression of NF-κB regulated genes that code for cyclooxygenase-2, 5-lipoxygenase, histone deacetylase, and vascular endothelial growth factor.

These and other reports on the effects of combined treatments of ACA and other substances are summarized in Table 3.

These findings may provide new insights for the use of novel combination therapies against diseases, especially carcinogenesis.

Structure-activity relationships of ACA

The relationships between structure and activity of ACA have been investigated in several experimental systems. Murakami *et al*. investigated the structure-activity relationship of ACA in a test for inhibition of tumor promoter teleocidin B-4-induced Epstein-Barr virus (EBV) activation in Raji cells by using 16 derivatives of ACA. As a result, they demonstrated that the structural factors which regulate its activity were (1) the two acetyl groups at positions 1' and 4. They also confirmed that (2) the unsaturated double bond between positions 2' and 3' of ACA are essential for ACA activity; and (3) the configuration at position 1' of ACA does not affect its activity [55]. Matsuda *et al*. examined the relationships in an inhibitory test to elucidate the

secretion of β-hexosaminidase, a marker of antigen-IgE-mediated degranulation in RBL-2H3 cells [40], and its inhibitory effect on nitric oxide (NO) production in lipopolysaccharide-activated mouse peritoneal macrophages [56]. Based on the effects of different compounds in the β-hexosaminidase release pathway, the 1'- and 4-acetoxyl groups of ACA and the 2'-3' double bond enhanced its activity.

For the inhibitory effects of ACA on NO production, Matsuda *et al*. clarified the following structure-activity relationships: (1) the para or ortho substitution of the acetoxyl and 1-acetoxypropenyl groups at the benzene ring is essential; (2) the S configuration of the 1'-acetoxyl group is preferable; (3) the presence of the 3-methoxyl group and the loss of the 2'-3' double bond by hydrogeneration reduce ACA activity; (4) the substitution of the acetyl groups with propionyl or methyl groups reduces ACA activity; and (5) the lengthening of the carbon chain between the positions 1' and 2' reduces ACA activity.

Azuma *et al.* studied the structure-activity relationship to elucidate the apoptotic activity of ACA in HL-60 cells human leukemia. As a result, they demonstrated that (1) the two acetyl groups at positions 1' and 4' of ACA and (2) the unsaturated double bond between positions 2' and 3' of ACA are essential for ACA activity, but (3) the configuration at position 1' of ACA does not affect its activity [57].

Liu *et al.* examined the structure-activity relationships of ACA using fission yeast expressing nuclear export signal of Rev, an HIV-1 viral regulatory protein. Based on their findings, (1) para substitution of the acetoxyl and 1'-acetoxypropenyl groups at the benzene ring is essential, (2) linear ethyl and propyl chain carbonates are more active than branching chain carbonates, and (3) substitution of the acetoxyl groups with alkylcarbomate groups prevents or reduces ACA activation [58].

These results demonstrate that the structural factors regulating the activity of ACA are: (1) the acetyl group at position 1'; (2) the acetyl group at position 4; and (3) the unsaturated double bond between positions 2' and 3' (Figure 2).

SUMMARY

ACA is found in *Alpinia galanga* and *Alpinia conchigera*, which are often used as traditional spices in cooking, especially in Thailand and Indonesia.

A quarter-century ago, Kondo *et al*. discovered the anticancer effects of ACA when they inhibited tumor promoter-induced EBV activation which was employed as an *in vitro* assay system. Since then, many researchers have investigated the anticancer effects of ACA in many experimental systems. Recent studies have shown that the combination with ACA and other substances exhibits synergistical effects on anticancer activities. These findings may give new insights for using novel combination therapies against cancer.

Many *in vitro* and *in vivo* researches have shown that ACA has several biological and pharmacological effects, including antimicrobial, antiallergy, anti-inflammatory, anti-obesity, and anti-asthmatic activities and gastroprotective effects. Recently, we demonstrated that ACA has protective effects against dementia [48]. Based on prior findings, these pharmacological effects of ACA might be mediated by the activation of AMPK.

Altogether, these findings indicate that ACA may be beneficial for preventing lifestyle-related diseases. However, more studies should be carried out to examine the clinical effect of ACA to obtain a better understanding of its potential.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interest exists.

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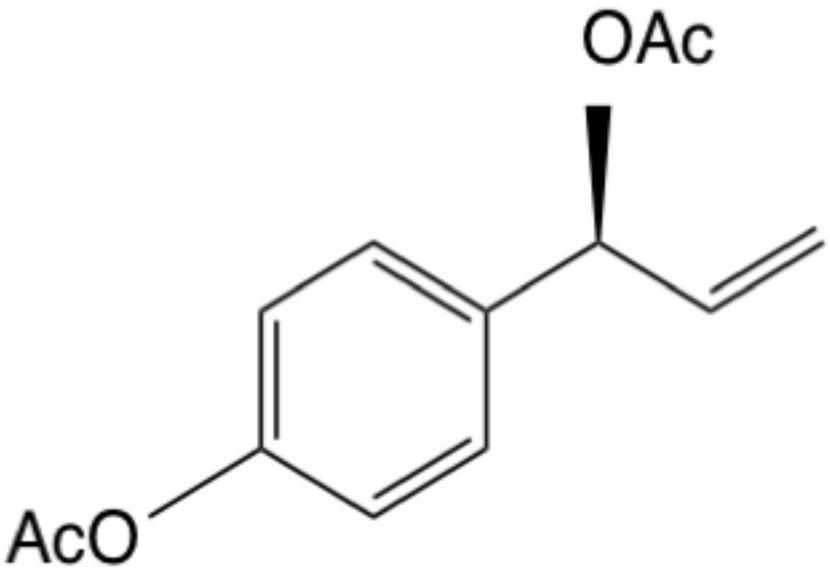

Figure 1. The structure of ACA.

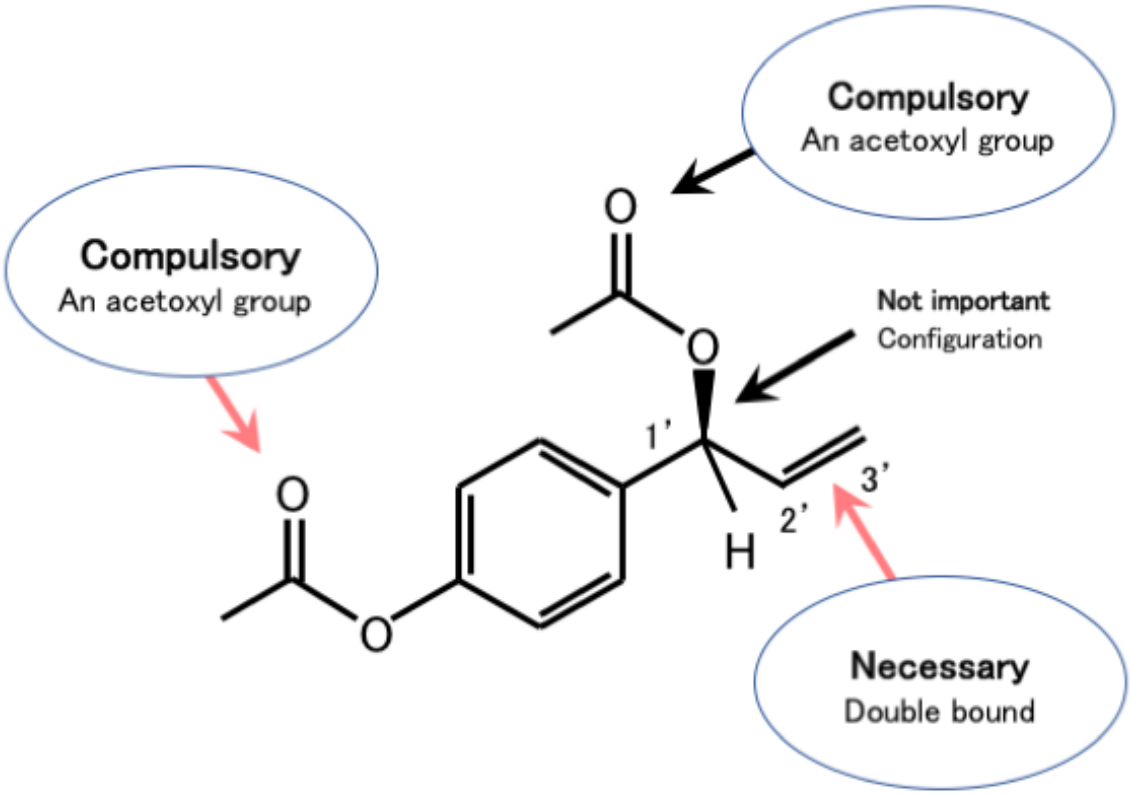

Figure 2. Structural factors regulating the activity of ACA.

Table 1. Anti-tumor activity of ACA.

Authors [References]	*In vitro* experiments	*In vivo* experiments	Comments on the effects of ACA
Kondo *et al.*, 1993 [1] and Ohigashi *et al.*, 1994 [2]	Epstein-Barr virus activation in Raji cells		Tumor promoter-induced Epstein-Barr virus activation ↓
Ohnishi *et al.*, 1996 [3]		4-nitroquinoline 1-oxide-induced oral carcinogenesis in male F334 rats	BrdU-labeling index ↓ Tongue polyamine levels ↓
Murakami *et al.*, 1996 [4]	Differentiated HL-60 cells	Two-stage carcinogenesis experiment in ICR mouse	The average number of tumors per mouse ↓ The ratio of tumor bearing mice ↓ TPA-induced superoxide generation in HL-60 cells ↓
Tanaka *et al.*, 1997 [5]		Azoxymethane-induced rat colon carcinogenesis	Significant reduction in the frequency of aberrant crypt foci ↓ Ornithine decarboxylase activity ↓ Blood polyamine content ↓
Kobayashi *et al.*, 1997 [6]		Endogenous rat liver carcinogenesis by feeding of choline-deficient and L-amino acid-deficient diet	Putative preneoplastic, glutathione S-transferase placental form-positive; lesions in the liver ↓ The levels of 8-hydroxyguanine, a parameter of oxidative DNA damage ↓
Miyauchi *et al.*, 2000 [7]		N-nitrosobis (2-oxopropyl)amine (BOP) - initiated hamster tumorigenesis model	BOP-induced cholangiocarcinogenesis ↓
Kawabata *et al.*, 2000 [8]		N-nitrosomethylbenzylamine-induced esophageal tumorigenesis in male F344 rats	Cell proliferation in the esophageal epithelium ↓ Blood polyamine contents ↓
Mori *et al.*, 2001 [9]	Human colorectal cancer cells (Colo 320)		The generation of apoptosis and the inhibition of cell proliferation ↑
Ito *et al.*, 2004 [10]	NB4 promyelocytic leukemia cells		Production of reactive oxygen species ↑ Mitochondrial transmembrane potential ↓ activation of caspase-9 ↑, Apoptosis ↑ Fas-mediated apoptosis by inducing of caspase-8 activity ↑ ACA induces apoptosis via independent dual pathways
Ito *et al.*, 2005 [11]	Multiple myeloma cells	RPMI8226-transplanted NOD/SCID mice	The nuclear expression of NF-kappa B ↓ G0-G phase cell cycle arrest ↑ Caspase 3, 9, and 8 activities ↑, Apoptosis ↑ Tumor weight in RPMI8226-transplanted NOD/SCID mice ↓
Ichikawa *et al.*, 2005 [12]	Human chronic myeloid leukemia (KBM-5), lung adenocarcinoma (H1299), human T cell lymphoma (Jurkat), human embryonic kidney carcinoma (A293), and MCF-7 (human breast adenocarcinoma (MCF-7) cells		NF-kappa B activation and its regulated gene expression ↓ I kappa B alpha kinase activation ↓
Batra *et al.*, 2012 [13]		Skin tumor promotion model in K5.Stat3C mice	Skin tumorigenesis ↓, Phospho-p65 NF-kappa B activation ↓
Ito *et al.*, 2005 [14]	Multiple myeloma cells		The expression of both TNF-related apoptosis-inducing ligand/Apo2 ligand (TRAIL/Apo2L) and TRAIL receptor death receptor 5 ↑

Campbell *et al.*, 2007 [15]	Human breast carcinoma cells (MCF-7, MDA-MB-231)		Activation of caspase-3 ↑
Moffatt *et al.*, 2000 [16]	Ehrlich ascites tumor cells		Intracellular polyamines ↓ Ornithine decarboxylase activity ↓ Spermidine/spermine N1-acetyltransferase ↑ Caspase-3 like protease ↑ Apoptosis ↑
Xu *et al.*, 2008 [17]	Mouse Ehrlich ascites tumor cells		(1'S)-ACA and its enantiomer inhibit tumor cells, G1 phase of the cell cycle in (S)-ACA ↑ Phosphorylation of retinoblastoma in (S)-ACA ↓ Phosphorylation of p27 (kip1) in (S)-ACA ↓ G2 phase of the cell cycle in (R)-ACA Phosphorylation of retinoblastoma in (R)-ACA ↑ Phosphorylation of p27 (kip1) in (R)-ACA ↑
Higashida *et al.*, 2009 [18]	Mouse Ehrlich ascites tumor cells		Apoptotic cell death was reversed by the addition of N-acetylcysteine or glutathione ethylester GSH levels ↓ γ-Glutamyl cysteine levels ↑
Moffatt *et al.*, 2002 [19]	Ehrlich ascites tumor cells		Tyrosine phosphorylation of proteins of 27 and 70 kDa ↑ Cellular glutathione and protein SH group ↓
Unahara *et al.*, 2007 [20]	Mouse Ehrlich ascites tumor cells		Accumulation of cells in the G1 phase, DNA synthesis ↓ Hyperphosphorylated retinoblastoma protein ↓ Phosphorylated p27 (kip1) ↓ Nuclear localization of p27 (kip1) ↑
Xu *et al.*, 2010 [21]	Mouse Ehrlich ascites tumor cells		Correlations were found among the decrease in cell viability, intracellular GSH, and the activity of glutathione reductase. The structure-activity relationship of ACA
Baradwaj *et al.*, 2017 [22]	Colorectal adenocarcinoma (SW480)		DNA damage ↑, mitochondria depolarization ↑, apoptosis ↑, G0/G1 arrest ↑, Expression of p21 ↑, cyclin D ↓
Pang *et al.*, 2011 [23]	(1) Human umbilical vascular endothelial cells (HUVECs) and (2) human prostate cancer cells (PC-3)	(3) Human prostate cancer PC-3 xenografts studies in mice	Proliferation, migration, adhesion, and tubulogenesis in (1) ↓ Cell viability, angiogenic factor production, and dual Sre/FAK kinases in (2) ↓ In (3), tumor volume and tumor weight ↓, levels of Src, CD31, VEGF and Ki-67 ↓
Phuah *et al.*, 2013 [26]	Human cervical carcinoma cells (Ca Ski)		Synergistic effects with the combination of ACA and cisplatin, A total of 25 miRNAs including has-miR-138, has-miR-210, and has-miR-744 ↑
Wang *et al.*, 2013 [27]	Human head and neck squamous cell carcinoma cell line (HN4)		Apoptosis ↑, the expression of miR-23a ↓
Phuah *et al.*, 2017 [29]	Cervical cancer cells		Overexpression of RSU1 augmented antiproliferative and apoptosis-inducing effects of cisplatin
Phuah *et al.*, 2017 [28]	Human cervical cells (CaSki and SiHa)		miR-210 expression ↓, mothers again decapentaplegic homolog 4 (SMAD4) expression ↑

ACA, 10-acetoxychavicol acetate; GSH, glutathione; GST, glutathione-S-transferases; HUVECs, human umbilical vascular endothelial cells; miRNA, microRNA; NF-jB, nuclear factor jB; ROS, reactive oxygen species; TRAIL/Apo2L, tumor necrosis factor-related apoptosis-inducing ligand/Apo2 ligand.

Table 2. Physiological effects of ACA, except its antitumor activity.

Physiological effects	Targets	Effective concentrations of ACA	Authors [References]
1. Anti-obesity activity	Transient receptor potential cation channel subfamily V. member 1(TRPA1) which is involved in the reduction of visceral body fat and exhibits anti-obesity effect.	EC50: 0.16 μM	Narukawa *et al*., 2010 [31]
	Inhibition of adipogenesis in 3T3-L1 adipocytes and high fat-fed rats	5 μM for adipocytes and diet containing 0.02% ACA for rats	Ohnishi *et al*., 2012 [33]
2. Gastroprotective effects	Gatric mucosal lesions induced by pylorus ligation	5 mg/kg	Mistui *et al*., 1976 [37]
	Gastric mucosal lesions induced by ethanol or 0.6 M HCl and aspirin	0.5 - 5.0 mg/kg	Matsuda *et al*., 2003 [38]
3. Xenobiotic protection	Phase II enzymes (GST and NQO in the liver and colon)	Diet containing 100~500 ppm	Tanaka *et al*., 1997 [5]
	Phase II enzymes (GST and NQO in intestinal epithelial cells)	5 μM	Yaku *et al*., 2011 [39]
4. Antiallergic activity	Inhibition of the degradation and release of TNF-α and Il-4 in RBL-2H3 cells	IC50: 15 μM	Matsuda *et al*., 2003 [40]
	Bronchoalveolar lavage fluid cell counts and histopathological and immunohistochemical analyses in a mouse model of ovalbumin-induced asthma	25 mg/kg/day or 50 mg/kg/day	Seo *et al*., 2013 [42]
5. Antimicrobial activity	*Mycobacterium tuberculosis* (H37Ra)	MIC: 0.024 μg/mL	Phongpaichit *et al*., 2006 [43]
	Mycobacterium tuberculosis (H37Ra ATTC 25177 and H37Rv ATTC 27284)	MIC: 0.2 and 0.7 μg/mL, respectively	Warit *et al*., 2017 [44]
	Anti-plasmid activity against-multidrug resistant bacteria	SIC: 400 - 800 μg/mL	Latha *et al*., 2009 [45]
	Propionibacterium acnes	MIC: 62.0 μg/mL	Niyomkam *et al*., 2010 [46]
6. Proteasome enhancing activity	Proteasome activity in differentiated PC12 cells	2.5 - 5 μM	Yaku *et al*., 2018, [47]
7. Preventive effect of dementia	The maintenance of the cognitive performance of senescence-accelerated mice	Diet containing 0.02% ACA	Kojima-Yuasa *et al*., 2016 [48]

Il-4, interleukin-4; MIC, minimum inhibitory concentration; NQO, NAD(P)H:quinone oxidoreductase; TNF-a, tumor necrosis factor-a; TRPA1, transient receptor potential cation channel subfamily V, member 1.

Table 3. Effects of combination treatment with ACA and other substances.

Authors [References]	Combined treatments (Activity) (Experimental systems)	Effects	Comments
Phuah *et al.,* 2013 [26]	ACA + cisplatin (Antitumor) (Human cervical carcinoma cell lines)	Synergistic	25 miRNAs ↑ (has-miR-138, has-miR-210, has-miR-744 etc.)
In *et al.,* 2012 [51]	ACA +cisplatin (Antitumor) (Oral squamous cell carcinoma cells and human oral tumor xenograft nude (Nu/Nu) mice)	Synergistic	NF-κB ↓ cyclin D1 ↓
Yaku *et al.,* 2013 [53]	ACA + sodium butyrate (Antioxidant) (Phase II activity in intestinal epithelial cells (IEC 6))	Synergistic	NRF2 protein ↑ AMPK phosphorylation ↑ p53 acetylation ↑ phase II enzymes ↑
Kato *et al.,* 2014 [52]	ACA + sodium butyrate (Antitumor) (Cultured human hepatocellular carcinoma cells (Hep G2))	Synergistic	Intracellular ROS ↑ NADPH oxidase ↑ AMPK phosphorylation ↑
Arshad *et al.,* 2015 [54]	ACA + recombinant human a fetoprotein (Antitumor) (Human cancer xenograft athymic nude (Nu/Nu) mice)	Synergistic	NF-κB activation ↓ The expression of NF-κB regulated genes ↓

AMPK, AMP-activated protein kinase; NRF2, nuclear factor, erythroid 2 (NF-E2)-related factor-2.