

Anticarcinogenic Effects of α -Mangostin: A Review

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Key words

α -mangostin, Clusiaceae, *Garcinia mangostana*, molecular mechanism, pharmacokinetics, toxicology


received June 21, 2016
revised October 10, 2016
accepted October 16, 2016

Bibliography

DOI <http://dx.doi.org/10.1055/s-0042-119651>
Published online November 4, 2016 | *Planta Med* 2017; 83: 188–202
© Georg Thieme Verlag KG Stuttgart · New York | ISSN 0032-0943

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ABSTRACT

Cancer chemoprevention is a promising strategy taken to block, reverse, or retard carcinogenesis. α -Mangostin, a natural xanthone isolated from the pericarps of mangosteen, represents one of the most studied chemopreventive agents. This compound has been reported to interfere with all the major stages of carcinogenesis: initiation, promotion, and progression. A number of mechanisms have been proposed for its anticarcinogenic activities. This review summarizes the current knowledge on the mechanisms that contribute to the observed activity of α -mangostin related to (i) modulation of carcinogenic biotransformation and mitigation of oxidative damage, (ii) induction of growth arrest and apoptosis, (iii) suppression of angiogenesis and metastasis, and (iv) combination with clinical chemotherapy drugs enhancing their efficacy and decreasing the toxic side effects. In addition, pharmacokinetic and toxicological studies of α -mangostin have also been highlighted in this review. Despite an overwhelming amount of knowledge in preclinical studies, there was almost no translation of α -mangostin into the clinic. It is hoped that continuous extensive and profound research will lead to the application of α -mangostin from experimental studies to evidence-based, clinically applicable pharmacotherapy.

Introduction

Cancer is a major leading cause of death worldwide. The American Cancer Society reported that nearly 577 190 cancer patients died and more than 1.6 million new cases occurred in the year 2012 only in USA [1]. More than 70% of all cancer deaths have occurred in low- and middle-income countries. The global burden of cancer will continue to increase because of aging and growing urbanization as well as accumulated adoption of a cancer-associated lifestyle like smoking, sedentary habits, and westernized diets [2]. It is estimated that there would be about 22 million new cancer cases and more than 13 million deaths worldwide by 2030 according to the WHO report [3].

Cancer is a multifactorial disease typically occurring over an extended period beginning with an initiation phase followed by promotion and progression phases. During the carcinogenic process, a cell acquires essential alterations conferring the progressive transformation of normal cells into cancer cells. The cellular alterations include evading growth suppressors and apoptosis, self-sufficiency in growth signals, uncontrolled replicating potential as well as sustained angiogenesis, tissue invasion, and metastasis

[4]. Over the past decades, significant progress has been achieved in our understanding of the molecular biology of cancer. Nevertheless, this deadly disease has not been conquered yet. The contemporary clinical therapies, including radiotherapy, chemotherapy, surgery, and immunosuppression, offer limited benefits to cancer patients due to metastasis, chemoresistance, or toxicity issues [5, 6]. Obviously, there is an urgent need to develop alternative treatment modalities for cancer. Chemoprevention, referred to the utilization of nontoxic chemical entities to block, reverse, or retard carcinogenesis, has emerged as an alternative but a more pragmatic strategy for the management of cancer. This approach has been shown great success, and the United States Food and Drug Administration has approved ten agents for cancer prevention, such as the identification and development of tamoxifen and raloxifene for reducing the risk of breast cancer, valrubicin for treatment of preinvasive bladder cancer, and a variety of topical and systemic agents that effectively treat preinvasive neoplastic lesions of the skin [7]. Epidemiological and experimental studies suggest that regular consumption of fruits and vegetables is a relatively practical strategy to reduce the risk of cancer [8]. Many non-nutritive substances derived from a plant-based diet, such as resveratrol, curcumin, sulforaphane, genistein, epigallocatechin-

ABBREVIATIONS

ACF	aberrant crypt foci
AIF	apoptosis inducing factor
AMPK	AMP-activated protein kinase
AOM	azoxymethane
aSMase	acid sphingomyelinase
BCAC	β -catenin accumulated crypts
Bmi-1	B-cell-specific moloney murine leukemia virus insertion site 1
CAT	catalase
CDKs	cyclin-dependent kinases
COX	cyclooxygenase
CRP	C-reactive protein
DF	dysplastic foci
DMBA	7,12-dimethylbenz[α]anthracene
DSS	dextran sulfate sodium
ER	endoplasmic reticulum
EMT	epithelial-mesenchymal transition
FAK	focal adhesion kinase
GLI1	glioma-associated oncogene homolog 1
GPx	glutathione peroxidase
GSH	glutathione
HO-1	heme oxygenase-1
iNOS	inducible nitric oxide synthase
LDL	low-density lipoprotein
LKB1	liver kinase B1
MMP	matrix metalloproteinase
mTORC1	mTOR complex 1
PARP	poly (ADP-ribose) polymerase
PCNA	proliferating cell nuclear antigen
P-gp	P-glycoprotein
PMA	phorbol 12-myristate-13-acetate
PSCs	pancreatic stellate cells
PVP	polyvinylpyrrolidone
SDF-1	stromal cell-derived factor-1
SIRT-1	sirtuin 1
TIMP1	tissue inhibitor of metalloproteinase-1
TPA	phorbol 12-otetradecanoate-13-acetate
VEGF	vascular endothelial growth factor
VEGFR2	vascular endothelial growth factor-2

3-gallate (EGCG), gingerol, and caffeic acid phenyl ester, commonly termed “phytochemicals”, have been identified as promising cancer chemopreventive agents [9]. According to the conventional classification originally proposed by Lee Wattenberg, these chemopreventive agents are generally classified into two main categories: (i) blocking agents that prevent the occurrence of the damage induced by a carcinogen in the initiation phase, and (ii) suppressing agents that control or reverse the damage in the promotion or progression phase [10]. In addition, some agents that intervene in all three stages of carcinogenesis are classified into both categories, and are considered as more promising molecules for cancer chemoprevention due to their multiple interventions.

Mangosteen (*Garcinia mangostana* L.) belongs to the family Clusiaceae and is known as “the queen of fruits” for its delicious taste. The pericarps of mangosteen have long been used in different countries (Malaysia, Indonesia, The Philippines, Sri Lanka, Thailand, etc.) for medicinal purposes, including treatment of abdominal pain, dysentery, diarrhea, suppuration, infected wound, and chronic ulcer [11]. Recently, numerous dietary supplements, beverages, and food products manufactured from mangosteen fruits are increasingly popular because of their proposed role in improving human health [12, 13]. α -Mangostin (α -MG; ► Fig. 1), the most abundant xanthone derived from the pericarps of mangosteen (78% content), is one of the most studied chemopreventive phytochemicals. α -MG has been reported to possess a variety of pharmacological properties, such as antioxidant, antiinfective, anticarcinogenic, and antidiabetic activities, as well as neuroprotective, hepatoprotective, and cardioprotective properties, among which the anticarcinogenic activity is the most promising [14, 15]. A great deal of evidence from *in vitro* and *in vivo* studies demonstrated that α -MG works on all of the major stages of tumor growth: initiation, promotion, and progression. α -MG acts as a blocking agent by the modulation of enzymes involved in the metabolic activation and excretion of carcinogens, resistance to oxidative damage, and attenuation of inflammatory response, while the inhibition of cell proliferation through modulating cell cycle regulatory machinery, induction of apoptotic effects on damaged and transformed cells, and blockage of angiogenic and metastatic processes of tumor cells contribute to its potential as a suppressing agent. A remarkable progress in interpreting the multiple mechanisms behind the anticarcinogenic activities of α -MG has been achieved over the past decades. Understanding how α -MG exerts its effects might provide new therapeutic strategies or avenues for the design and development of more potent drugs to efficiently control cancer. In this review, we examine the current knowledge on the mechanism-based *in vitro* and *in vivo* studies about the anticarcinogenic activities of α -MG, and integrate these modes of action as potential examples to provide how α -MG could prevent carcinogenesis.

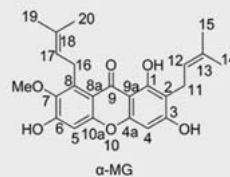
Antitumor Activity of Mangostin in Animal Models

The direct evidence of chemopreventive effects of dietary phytochemicals always comes from cell culture systems and animal models, as it is difficult to assess the real impact on human health [16]. In particular, animal models may provide more convincing evidence, and α -MG has been reported to exert anticarcinogenic activities in several animal models of experimentally induced carcinogenesis (► Table 1). Arakaki et al. [17] established a mouse colon carcinogenesis model employing AOM as the initiating carcinogen and DSS as the promoting agent. They observed that dietary α -MG significantly decreased the PCNA positive index of colon tumors and increased the caspase-3 positive index of them. In another similar study conducted by Nabandith et al. [18], a short-term dietary administration of crude α -MG to rat models of 1,2-dimethylhydrazine-induced colon carcinogenesis showed a marked reduction of the formation of ACF in the colon and de-

crease of both DF and β -catenin accumulation in the crypts in a dose-dependent fashion. These results suggest that α -MG has potential as a chemopreventive agent by inhibition of cell proliferation and induction of apoptosis against preneoplastic cells. Besides, several investigators utilized nude mice models to assess the chemopreventive efficacy of α -MG. The studies were mostly performed in nude mice ectopic xenograft models, where α -MG exhibited significant inhibitory effects against the growth of (i) human colorectal adenocarcinoma HCT116 and HT-29 cells through the induction of apoptosis and inhibition of angiogenesis [19,20], (ii) prostate cancer 22Rv1 cells through arresting cell cycle [21], (iii) human hepatoma SK-Hep-1 cells and tongue mucoepidermoid carcinoma YD-15 cells through the induction of apoptosis [22,23], and (iv) glioblastoma GBM8401 cells through the induction of autophagic death [24]. In addition, for pancreatic cancer, α -MG not only markedly inhibited pancreatic cancer ASPC1 cell-derived ectopic xenograft tumors [25,26], but also PL-45 cell-derived orthotopic xenograft tumors [25], which can better maintain the original biological characteristics of tumor cells and have a relatively higher clinical relevance. Importantly, besides the inhibitory effects on the growth of primary tumors, α -MG has also shown significant antimetastatic effects in animal models. Shibata et al. [27] successfully developed a metastatic cancer model by implanting murine mammary adenocarcinoma BJMC3879luc2 cells into syngeneic BALB/c mice, which induces a metastatic spectrum similar to that seen in human breast cancer. Using this model, they confirmed that α -MG significantly attenuated the growth of tumors and the multiplicity of lymph node metastasis. Given that lymph node involvement is the most important prognostic factor in breast cancer patients, the antimetastatic activity of α -MG detected in such a model may be of great significance in future clinical applications. However, the underlying mechanism remains unclear. It is well known that most solid tumors rely on angiogenesis to support their localized growth and metastatic dissemination, and the vascular density of a tumor is closely associated with its metastatic potential. Hence, the reduction of microvessel density and suppression of tumor angiogenesis by α -MG in this context may partially explain its suppressive effects on metastasis. Certainly, before a definite impact of α -MG in this regard can be ascertained, deeper investigations will be required.

Mangostin as a Blocking Agent: Blocking Cancer Initiation

Cancer initiation occurs as a consequence of rapid and irreversible attacks to cells. The attack may be due to the initial exposure to or uptake of a carcinogen and the subsequent stable genotoxic damage caused by its metabolic activation [28]. Other major causes include oxidative stress [29] and chronic inflammation [30]. The following section will discuss how α -MG protects cells from the damage induced by metabolically activated carcinogens, oxidative stress, or chronic inflammation, thereby obstructing the initiation of carcinogenesis.



► Fig. 1 Chemical structure of α -MG.

Modulation of phase I and phase II enzymes

Many chemical carcinogens are metabolized by a process termed biotransformation. This process includes oxidative metabolism by phase I enzymes, especially those that belong to the cytochrome P450 (CYP) superfamily, and conjugation with polar groups by a series of phase II enzymes [e.g., glutathione-S-transferase, UDP-glucuronosyltransferase, sulfotransferase, NAD(P)H: quinone oxidoreductase]. However, in the absence of phase II enzymes, this process may result in the production of excessive amounts of ROS and other active substances that assault cellular DNA, and thus procarcinogens are converted into carcinogens. Chemopreventive strategies include the inhibition of phase I enzymes responsible for activating carcinogens and induction of phase II enzymes that conjugate these activated compounds with endogenous ligands, thus contributing to their elimination [31].

Jung et al. [32] reported that α -MG reduced the insurgence of DMBA-induced preneoplastic lesions in an *ex vivo* mouse mammary organ culture assay. Given that DMBA bioactivation requires phase I enzymes CYP1A1, CYP1A2, and CYP1B1 [33], it was reasonable to speculate that α -MG repressed the initiation of carcinogenesis partly due to its inhibiting phase I enzymes. CYP19 is another member of the CYP family, alternatively known as aromatase, which is a rate-limiting enzyme in the biosynthesis of estrogen. Using an enzyme-based microsomal aromatase inhibition assay, Balunas et al. [34] found that α -MG inhibited aromatase in a dose-dependent manner. Since estrogen plays a vital role in the development and progression of hormone-responsive breast cancers, α -MG's suppressive effect on CYP19/aromatase indicates its potential as a chemopreventive or a chemotherapeutic agent in mammary carcinogenesis. Importantly, it was reported that in a concentration- and time-dependent way, α -MG treatment increased the protein level and activity of HO-1, an important cytoprotective protein induced during the phase II response [35]. Currently, however, studies regarding the effects of α -MG on phase I and II enzymes and the involved signaling pathways are limited, which warrant further investigations.

Antioxidant activity

ROS/RNS is generated by various endogenous and exogenous pathways, such as the mitochondrial respiratory chain, inflammatory processes, lipid peroxidation, UV irradiation, and environmental pollutants [36]. Under normal conditions, cells maintain ROS/RNS at proper levels by the antioxidant action of enzymatic antioxidants, such as SOD, CAT, and GPx, as well as nonenzymatic antioxidants, such as GSH and uric acid [37]. When this balance is

► **Table 1** Antitumor activity of α -MG in animal models.

Animal model	Used dosage	Mechanisms/effects	Reference
1,2-DMH-induced colon cancer in F344 rats	Dietary administration of 0.02% or 0.05% crude α -MG (77.8%) for 5 weeks	Inhibition of induction and development of ACF; decrease in DF and BCAC; lowering PCAN in colon	[17]
AOM- and DSS-induced colon cancer in CD1 mice	Dietary administration of 200 or 500 ppm α -MG for 16 weeks	Decreased multiplicities of neoplasms; decreased PCAN positive index in colon tumor; increased caspase-3 positive index	[18]
Human colon cancer HCT116 cells xenograft tumor in athymic NCR nu/nu nude mice	Dietary administration of 0.25% or 0.5% mangosteen pericarp extract (α -MG 81%) for 20 days	Inhibition of tumor growth via direct cytotoxicity on the tumor cells and/or reduction the number of intratumor blood vessels	[19]
Human colon cancer HT-29 cells xenograft tumor in BALB/c nu/nu mice	Dietary administration of 900 mg/kg α -MG for the 5 weeks	Reduction of tumor mass; decrease in concentrations of Bcl-2 and β -catenin in tumors	[20]
Human prostate cancer 22Rv1 cells xenograft tumor in athymic nu/nu mice	Oral gavage of 100 mg/kg/BW α -MG five times weekly	Inhibition of tumor growth via targeting cyclin D1 and CDK4	[21]
Human hepatoma SK-Hep-1 cells xenograft tumor in immunodeficient BALB/c male mice	Intraperitoneal administration of 8 mg/kg/BW α -MG daily for 42 days	Inhibition of tumor growth; induction of mitochondria apoptotic pathway via inactivation of p38 MAPK	[22]
Human tongue mucoepidermoid carcinomanude YD-15 cells xenografts tumor in nude mice	Intraperitoneally administration of 10 or 20 mg/kg/BW α -GM five times weekly for 22 days	Decrease of tumor weight; decrease of Ki-67 expression; induction of mitochondria apoptotic pathway via inactivation of p38 MAPK and ERK1/2	[23]
Human malignant glioblastoma GBM8401 cells xenograft in nude Balb/cA-v (v/v)	Intraperitoneal administration of 2 mg/kg/BW α -MG daily for 28 days	Inhibition of tumor growth via increasing phosphorylation of AMPK and induction of autophagy	[24]
Secondary human pancreatic cancer ASPC1 cell-derived ectopic xenograft tumors in nude mice	Intraperitoneal administration of 6 mg/kg/BW α -GM five times weekly for 8 weeks	Inhibition of Ki-67 and PCNA in tumor tissues	[25, 26]
Primary human pancreatic cancer PL-45 cell-derived orthotopic xenograft tumors in nude mice	Intraperitoneal administration of 6 mg/kg/BW α -GM five times weekly for 8 weeks	Inhibition of Ki-67 and PCNA in tumor tissues	[25]
Murine mammary adenocarcinoma BJMC3879luc2 xenograft tumor in syngeneic BALB/c mice	10 or 20 mg/kg/BW α -GM daily via the mini-osmotic pumps for six weeks	Suppression of tumor growth and lymph node metastasis via apoptotic cell death, reduction of phospho-Akt, and microvessel density	[26]

disturbed, cellular defenses can be overwhelmed, and the cell is injured by oxidative modifications of proteins and carbohydrates, DNA strand nicks, and lipid peroxidation. It has been proven that α -MG possesses protective effects in models where oxidative stress and antioxidant deficit are major players, such as rats with isoproterenol-induced myocardial infarction [38,39], Langendorff-reperfused hearts [40], and rat brain tissues exposed to different toxic insults [41]. The underlying mechanisms include (i) direct free radical scavenging, (ii) modulation of oxidative stress-related enzymes, and (iii) attenuation of inflammation.

α -MG was firstly proven to have antioxidant activity using the ferric thiocyanate method [42,43]. Thereafter, Williams et al. [44] found that α -MG served as a free radical scavenger to inhibit LDL oxidation induced by copper or peroxy radical. Consistently, Mahabusarakam et al. [45] reported that α -MG and its synthetic derivatives inhibited the decrease of α -tocopherol consumption in copper-induced LDL oxidation, and the structural modifications of α -MG differently affected the antioxidant activity: (i) substitution of C-3 and C-6 hydroxyl groups with aminoethyl increased

the activity, and (ii) substitution of C-3 and C-6 hydroxyl groups with methyl, acetate, propanediol, or nitrile reduced the activity. There is substantial evidence supporting the capacity of α -MG to scavenge superoxide anion ($O_2^{\cdot-}$), hydroxyl radicals ($OH\cdot$), and H_2O_2 [46–49]. On the other hand, α -MG was shown to possess peroxynitrite ($ONOO^-$) scavenging activity using authentic and 3-morpholinysydnonimine-derived peroxynitrite methods [32, 46]. Moreover, studies carried out in several cell/tissue models have confirmed the effectiveness of α -MG in counteracting lipoperoxidation and oxidative stress-mediated damage by its ability of quenching free radicals. For example, in cultured Chinese hamster lung cells exposed to paraquat (a $O_2^{\cdot-}$ generator) and $NaNO_2$, α -MG treatment demonstrated potent antigenotoxic effects [50]. Also, it has been found that in brain tissue exposed to different toxic insults, α -MG exerts a robust antiperoxidative effect [41]. However, such activity could lead to the formation of semiquinone radicals and quinones. For the sake of security, it has to be taken into account that a high concentration of α -MG might exert prooxidant activities, potentially favoring toxic effects and tissue dam-

ages. Indeed, this compound was observed to protect mitochondria from peroxidative damage, but at a high concentration, it disrupted the mitochondrial energy metabolism and made it prone to permeability transition by triggering oxidative stress [51].

Intervention with the cause of oxidative stress by inhibiting enzymes that generate ROS is another approach of antioxidant strategy. aSMase, a member of sphingolipid catabolic enzymes, catalyzes the breakdown of sphingomyelin to generate ceramide [52]. Accumulating evidence demonstrated that aSMase activation and ceramide production are upstream signals of ROS, which trigger the activation of ROS-generating enzymes, including NADPH oxidase, xanthine oxidase, NO synthase, and the mitochondrial respiratory chain [53–55]. Therefore, the inhibition of aSMase activity and ceramide production might be an effective strategy to disturb the production of ROS. In a study by Okudaira et al. [56], α -MG was identified to effectively inhibit the activity of aSMase derived from bovine brain and the production of ceramide, suggesting its potential in hampering the process of ROS production. Recently, our research group is devoted to searching for effective aSMase inhibitors. We have firstly constructed 3D-pharmacophore based on ligands and acquired a great array of mangostin derivatives, some of which were comparable to or more potent than α -MG [57]. Besides inhibiting ROS-generating enzymes, α -MG also exerted potential in the modulation of antioxidant enzymes. For example, in rat brain synaptosomes exposed to 3-nitropropionic acid, α -MG increased GPx activity and transiently depleted GSH levels [58]. Pretreatment with α -MG to rats diminished the decrease of SOD, CAT, and GPx in isoproterenol-induced myocardial infarction, rendering them to recover to normal levels, and concomitantly decreased lipid peroxidation levels [38].

A sustained production of ROS/RNS contributes to the pathological consequences of chronic inflammation. Activated inflammatory cells (e.g., neutrophils, monocytes, and macrophages), in turn, give rise to a variety of ROS/RNS (e.g., $O_2^{\cdot-}$, NO, ONOO $^-$, and $\cdot OH$), leading to various genetic/epigenetic changes triggering the initiation of carcinogenesis [59, 60]. iNOS is well known to be implicated in the excessive production of NO in response to immunological or inflammatory stimuli. In RAW 264.7 macrophages activated with LPS, α -MG remarkably reduced the levels of iNOS expression and NO production, indicating that it possesses anti-inflammatory and antitumorigenic potential [61, 62]. COX-2 is the rate-limiting enzyme in the conversion of arachidonic acid to prostaglandins. It is induced in many types of cells by inflammatory mediators and various stimuli (e.g., oncogenes, mitogens, tumor promoters, and growth factors). COX-2-dependent reactions can generate ROS during the conversion of arachidonic acid to prostaglandin G₂, causing direct oxidative damage to DNA [63]. Moreover, the products prostaglandins are thought to be a causative factor of cellular injury and may ultimately result in carcinogenesis. In breast cancer MDA-MB-231 cells treated with α -MG and stimulated with LPS, the induction of COX-2 was markedly attenuated in a dose-dependent manner both at the transcriptional and translational levels [64]. Besides, Navya et al. [65] investigated the molecular interaction mechanisms of α -MG with the two enzymes iNOS and COX-2 by utilizing computer docking tools. The results suggested that α -MG was a ligand against COX-2 and iNOS, respectively, and bound with them through the hy-

drogen bond interaction. Further experimental tests are required to determine whether this compound can directly act on COX-2 and iNOS or not. As a mechanism upstream to iNOS and COX-2 inhibition, α -MG was shown to activate SIRT-1, a nicotinamide adenosine dinucleotide-dependent histone deacetylase. SIRT-1 physically interacts with the p65/RelA subunit of NF- κ B and suppresses NF- κ B-driven gene transcription by deacetylating p65/RelA at lysine 310 [66]. The overproduction of ROS inhibits SIRT-1 activity by initiating oxidative modifications on its cysteine residues, thereby enhancing NF- κ B signaling resulting in inflammatory responses. As reported in a recent study, α -MG was observed to abrogate the acetylation of p65/NF- κ B and NF- κ B-regulated proinflammatory gene expressions, including COX-2 and iNOS, by activating SIRT-1 in the U937 cell line [67].

Mangostin as a Suppressing Agent: Suppressing Cancer Promotion and Progression

In the cancer promotion phase, cells with a dysfunction of key cellular proliferation control and apoptosis regulatory proteins persist, actively replicate, and accumulate to originate a focus of preneoplastic cells. Subsequently, the preneoplastic cells transform into neoplastic ones with the acquisition of angiogenic properties as well as invasive and metastatic potential, entering into the stage of cancer progression. Therefore, the strategies that can induce cell cycle arrest and/or apoptosis, disrupt angiogenesis, or prevent tumor cells escaping from the original location and invading other tissues could be beneficial for cancer prevention [68]. α -MG has been shown to have antiproliferative, proapoptotic, antiangiogenic and antimetastatic activities against a wide range of cancer cell types by modulating various aberrant molecules and signal transduction pathways.

Induction of cell cycle arrest

Defects in cell cycle regulation are major causes of the abnormal proliferation of cancer cells, among which the deregulation of the G₁/S transition is especially common in human cancers [69, 70]. α -MG has been shown to modulate the major cell cycle mediators at micromolar concentrations, causing a blockage of the G₁/S transition, resulting in a G₁-phase cell cycle arrest in many cancers, such as prostate cancer [71], melanoma [72], breast cancer [73], and pancreatic cancer [74]. Its antiproliferative activity involved the downregulation of cell cycle-related molecules CDKs 4/6, cyclins D1/D3/E, and hyperphosphorylated Rb, and the induction of cyclin-dependent kinase inhibitors p53, p21, and p27. For example, Johnson et al. [71] demonstrated that α -MG effectively blocked the G₁ phase progression by upregulating the protein expression of p27KIP1 and downregulating that of cyclins D1 and D3, phosphorylated Rb, and cyclin E in human prostate cancer 22Rv1 cells. In this study, the investigators also found that α -MG could directly inhibit the activity of CDK4 as shown in a cell-free biochemical kinase assay, and generated hypothetical binding arrangements between CDK4 and α -MG by employing molecular modeling. It is tempting to speculate that this compound is

able to fit within the deep and narrow ATP binding pocket of CDK4 due to its planar structure and hydrophobic isoprenyl groups along with neighboring hydrogen-bonding donors at C-1 and C-3. The tumor suppressor p53 mainly induces p21 under stress conditions in order to maintain the balance between cell growth and death in living systems, however, this “gatekeeper” is often inactivated by mutations that lead to unlimited cellular proliferation in various cancers [75]. Interestingly, in cells lacking wild-type p53, α -MG could still induce the expression of p21 and subsequent G1 phase arrest in a p53-independent pathway [72, 73]. In another interesting study, α -MG was found to inhibit the proliferation of colon cancer HCT116 cells in a p53-dependent manner, but the expression of p21CIP1 was proven unnecessary [76]. The investigators examined the underlying mechanism and provided evidence for the first time that α -MG arrested cells in the G1 phase by the induction of another tumor suppressor p16INK4A via activation of the p38 MAPK stress kinase pathway and consequent downregulation of Bmi-1. p16INK4A is commonly inactivated in cancer cells under the repressive effects of oncoprotein Bim-1. Activation of the p38 MAPK signaling pathway can lead to the phosphorylation and protein degradation of Bim-1, thereby terminating its negative effect on p16INK4A and restoring the p16INK4A-induced growth inhibition and cell cycle arrest [77]. This work provides new insights into the molecular basis of α -MG's antiproliferative activity outside its role in the p53-p21 axis. In other cancer cells, α -MG was reported to arrest the cell cycle at the S phase [78], G0/G1 phase [25, 79], or G2/M phase [80]. In addition, α -MG's antiproliferative activity might also be ascribed to its direct inhibition of DNA synthesis by diminishing DNA polymerases and topoisomerases. Dating back to 1997, α -MG was firstly found to exhibit inhibitory effects on DNA topoisomerases I and II. A recent study by Mizushima et al. [80] reported on its potent effects on both polymerases and topoisomerases. Moreover, α -MG treatment afforded a significant inhibition against topoisomerases, halting the cell cycle at the G2/M phase in HCT116 cells. Also, this study suggested that α -MG did not bind to double-stranded DNA acting as a DNA intercalating agent or as a template-primer substrate, but rather that this compound directly bound with the enzyme and inhibited its activity, as shown by the thermal transition analysis. In contrast, the classical inhibitors directly bind to DNA and subsequently indirectly suppress the activity of topoisomerase. Hence, α -MG might be a new type of topoisomerase inhibitor.

Induction of apoptosis

Apoptosis plays a critical role in eliminating the mutated and hyperproliferative neoplastic cells from the organism, and is therefore regarded as a major protective mechanism against the development of cancer. α -MG has been reported to induce apoptosis of various cancer cells in culture and implanted tumors in animal models via modulation of multiple proapoptotic and antiapoptotic signaling molecules [81, 82]. Importantly, it can selectively target cancer cells while having little influence on normal cells, indicating its potential in circumventing conventional chemotherapeutic-induced toxicity [83–85].

In general, there are two major apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial

pathway. The extrinsic pathway is induced through the binding of death ligands with death receptors on the cell surface leading to the activation of caspase-8. The intrinsic pathway is characterized by the loss of mitochondrial membrane potential, leading to the release of cytochrome c and caspase-9 activation [86]. Many studies suggested that α -MG induced apoptosis through both the extrinsic and intrinsic pathways as shown by the activation of caspases-8, -9 and -3, such as human hepatoma SK-Hep-1 cells [22], melanoma SK-MEL-28 cells [72], and breast cancer MDA-MB-231 cells [78]. Interestingly, Watanapokasin et al. [87] observed the upregulation of Fas and tBid occurring in human colorectal cancer COLO 205 cells. The proposed mechanism for α -MG-induced apoptosis was that upon α -MG treatment, the extrinsic pathway was activated, procaspase-8 was cleaved to caspase-8, and then further activated the cleavage of Bid to tBid; the tBid then translocated to mitochondria resulting in the activation of the mitochondrial apoptotic pathway [87]. The depletion of mitochondrial membrane potential, decrease in intracellular ATP, downregulation of proapoptotic Bcl-2 and upregulation of antiapoptotic Bax, release of cytochrome c from mitochondria into cytosol, activation of the caspase cascade, and enhancement of PARP cleavage were the main events involved in the mitochondrial apoptotic pathway induced by α -MG [23, 88]. However, this was not in agreement with the findings of Matsumoto et al. [89] who reported that α -MG directly targeted mitochondria and induced apoptosis as evidenced by the activation of caspases-9 and -3, but not caspase-8. This obvious conflict in the data may be because this compound exerted its activity by different pathways in different types of cancer cells. Caspases are common to both the extrinsic and intrinsic pathways, however, α -MG induced caspase-independent mitochondrial apoptosis with the release of a caspase-independent apoptogenic factor endonuclease-G from mitochondria in human colorectal cancer DLD-1 cells. Interestingly, this study also suggested that the miR-143/ERK5/c-Myc pathway was involved in α -MG-induced apoptosis [90].

α -MG induced apoptosis in various cancer cells is in a cell-type-specific manner, being p53-dependent in certain cells, while p53-independent in others. In human colorectal carcinoma HCT 116 cells, α -MG induced apoptosis by increasing p53 transcriptional activity followed by activating the ERK pathway [19]. Increased levels of p53 and Bax, and decreased Bcl-2 were detected in various head and neck squamous carcinoma cell lines when exposed to α -MG [91]. In addition, α -MG had the capability to inhibit the negative effect of MDM2 on p53, as a potential inhibitor of the p53-MDM2 interaction, restoring the p53-induced growth inhibition in breast cancer cell MCF-7 (with wt p53 and overexpression of MDM2). This finding provides a new perspective into the mechanism of action of α -MG in human cancer cells with wt p53 [92]. However, the α -MG-induced apoptotic pathway in breast cancer BJMC3879luc2 cells harboring a p53 mutation emerged through a p53-independent mechanism [93].

α -MG-treated MCF-7 cells displayed dose- and time-dependent downregulation of estrogen receptor α , along with the reduced expression of the estrogen-responsive pS2 gene, suggesting its apoptotic effect in estrogen receptor-positive breast cancer was at least partly mediated through an estrogen receptor-related pathway [94]. α -MG has been reported to decrease cell viability

and inhibit clonogenic survival through induction of autophagy, but not apoptosis, in glioblastoma GBM8401 cells by activation of the LKB1/AMPK pathway and subsequent inactivation of mTORC1 [24]. In chronic myeloid leukemia cell lines, inhibition of autophagy enhanced the α -MG-mediated cytotoxicity through promoting apoptosis [84]. This was not surprising because autophagy has been well characterized to play the Janus role, promoting tumor cell survival in the presence of certain stresses on one hand, but inhibiting the tumor cell growth on the other hand [95]. Recently, ER stress has been regarded as the third apoptotic pathway. α -MG was found to selectively promote ER stress so as to increase the apoptotic indices of prostate cancer cells, while exerting little influence on normal prostate epithelial cells [96,97].

Suppression of angiogenesis

Abnormal angiogenesis is necessary for tumor growth and metastasis. Thus, effective inhibition of angiogenesis may provide a crucial strategy for halting the process of carcinogenesis. It is well known that most solid tumors induce vascular proliferation by producing angiogenic factors, prominently VEGF [98]. VEGF can stimulate endothelial cell proliferation, migration, and subsequent differentiation to form new vessels. It exerts its angiogenic properties by binding with VEGFR (mainly VEGFR2), which is found at the surface of endothelial cells. This binding causes conformational changes of the receptors, followed by dimerization and autophosphorylation of the tyrosine residues, thereby activating downstream signaling, such as MAPK and PI3K/Akt pathways [99]. α -MG has been shown to reduce the expression of VEGF in T47D breast cancer cells *in vitro* [100] and pancreatic cancer ASPC1 cells-derived xenograft tumors in nude mice [26]. Hypoxia-activated secretion of VEGF-A and SDF-1, which are associated with angiogenesis and chemoattraction of cancer cells and endothelial cells, was abolished by α -MG [101]. In addition, α -MG inhibited VEGF-mediated biological effects by interfering with VEGF signaling pathways. Jittiporn et al. [102] found that α -MG dramatically and dose dependently suppressed VEGF-induced hyperpermeability, increased proliferation, migration, and tube formation in retinal endothelial cells, and limited vascular sprouting in the *ex vivo* aortic ring assay through suppressing VEGF-induced phosphorylation of VEGFR2 and ERK1/2 (a major downstream target of VEGFR2). Another study reported on its inhibitory effects on VEGF-induced proliferation, migration, and tubule formation of human umbilical vein endothelial cells [103]. This study also examined site-specific phosphorylation of VEGFR2 and revealed that α -MG inhibited ATP-induced phosphorylation of recombinant VEGFR2 at the Y1175. Taking these results together, it can be concluded that α -MG possesses dual roles, the decrease of the VEGF secretion from tumor cells and the direct action on endothelial cells. Such dual activities of α -MG, different from the single effect of other angiogenesis inhibitors, should rationally make α -MG elicit better antiangiogenic activity.

Inhibition of migration, invasion metastasis

The invasion of tumor cells through tumor-associated stroma and subsequent metastasis are the central events in neoplastic progression [104]. Excessive degradation of extracellular matrix mediated by proteolytic enzymes such as activated MMPs, especially

MMP-2 and MMP-9, is one of the hallmarks of tumor invasion and migration. α -MG could downregulate the expressions of MMP-2 and MMP-9 in a concentration-dependent manner, thereby blocking the invasion and metastasis of various cancers, including skin cancer [105], head and neck squamous cell carcinoma [106], lung adenocarcinoma [107], prostate carcinoma [108], and pancreatic cancer [109]. The underlying mechanisms were closely associated with the suppression of upstream kinase(s), such as MAPK family members (ERK, JNK, and p38) and PI3K/Akt, and the inactivation of downstream transcription factor(s), such as NF- κ B and AP-1. In addition, the study performed in PMA-treated highly metastatic human lung adenocarcinoma A549 cells further explored the upstream regulators, a new finding from which ERK1/2 inhibition and NF- κ B inactivation emerged through impeding the activation of α v β 3 integrin and phosphorylation of FAK [107]. TIMPs occur naturally within the extracellular matrix, which inhibit both pro- and active MMPs and provide a homeostatic environment in the matrix. The anti-invasive potential of α -MG against various pancreatic cancer cell lines was partly ascribed to the upregulation of TIMP1 and subsequent inhibition of MMP9 [25].

EMT is the process wherein epithelial cells acquire fibroblast-like properties and show decreased cell-cell adhesion and enhanced motility. Its occurrence during tumor progression was proven to be a major mechanism responsible for the invasiveness and metastasis of cancer cells. α -MG was found to block EMT progression of pancreatic cancer BxPc-3 and Panc-1 cells, as evidenced by the upregulation of epithelial marker E-cadherin and the downregulation of mesenchymal markers vimentin and N-cadherin in the control of the PI3K/Akt signaling pathway [73]. The action of inhibiting EMT progression further identified its inhibitory capacity on dissemination and invasion. Recent advances indicate that the tumor microenvironment plays a key role in tumor progression and invasion. A recent study conducted by Lei et al. [101] reported that PSCs and hypoxia exhibited a synergistic effect on accelerating pancreatic cancer EMT and invasion. Interestingly, α -MG had a protective effect against hypoxia in the pancreatic tumor-stromal interaction, including abolishing hypoxia-driven PSC activation, inhibiting EMT, and blocking cancer cell invasion, which was associated with its ability to inhibit hypoxia-induced ROS production as well as HIF-1 α stabilization and GLI1 expression.

Cytotoxic activity in combination with clinical chemotherapeutic drugs

Phytochemicals can synergize with classical chemotherapeutic drugs, increasing their efficacy and lowering the toxic side effects on normal cells, including α -MG. For example, α -MG exhibited a synergistic effect on 5-FU-induced growth inhibition of human colon cancer DLD-1 cells at lower concentrations (<5 μ M), decreasing the clinical dose of 5-FU, thereby lowering the systemic toxicity of 5-FU and increasing its therapeutic index [89]. α -MG protected renal epithelial cells from cisplatin-induced apoptosis via inhibition of p53 induction and ROS generation. On the other hand, it attenuated oxidative/nitrosative stress as well as inflammatory and fibrotic markers, offering renoprotective effects against cisplatin-induced injury in rats [110,111]. In addition, α -MG possessed protective effects against doxorubicin-induced

neurotoxicity by ameliorating oxidative damage and regulating proapoptotic and antiapoptotic proteins [112].

Inhibition of survival pathways

The following section will summarize studies that investigated the effects of α -MG on survival pathways. Activation of the MAPK (JNK, ERK1/2, and p38) or Akt signaling pathway is involved in a variety of oncogenic processes, including cell proliferation, anti-apoptotic cell death, angiogenesis, and metastasis [113,114]. Several studies have reported that α -MG inhibited the MAPK or PI3K/Akt pathway, thereby exerting its inhibition of cell growth and induction of apoptosis in cancer cells [115]. For example, α -MG induced mitochondria-mediated apoptosis of YD-15 tongue carcinoma cells through inhibiting phosphorylation of ERK1/2 and p38 MAPK in a dose-dependent manner [23]. Phosphorylated and total ERK, JNK, and Akt were downregulated in α -MG-treated SW1353 cells, but there were no changes in p38 MAPK [88]. In both mammary carcinoma cells in culture and tissues *in vivo*, α -MG significantly decreased the levels of phospho-Akt-Thr308 [27]. In addition, there is substantial evidence that α -MG restrained the process of invasion and migration in cancer cells by acting on the MAPK or Akt pathway. Indeed, α -MG could mediate the metastasis of human prostate cancer PC-3 cells through reducing the expression of MMP-2/9 and u-PA via the suppression of the JNK1/2 signaling pathway and inhibition of NF- κ B and AP-1 DNA binding activity [108]. In pancreatic cancer cell lines MIAPaCa-2 and BxPC-3, α -MG suppressed LPS-induced invasion and metastasis by inhibiting MMP-2/9 expression and increasing E-cadherin through inactivating the ERK signaling pathway [109].

Signaling pathways mediated via upstream kinases converge on divergent classes of transcription factors, such as NF- κ B and STAT3 [116]. Aberrant and sustained activated NF- κ B has been implicated in various stages of tumorigenesis and is found in a number of cancers. A number of natural chemopreventive agents have been reported to be potent inhibitors of NF- κ B, including α -MG. The carcinogens like PMA and TPA, as well as inflammatory agents like LPS and TNF- α are potent activators of NF- κ B, and α -MG could inhibit the activation of NF- κ B induced by these agents in a dose-dependent manner. In fact, α -MG not only suppressed the inducible form of NF- κ B, but also inhibited constitutively activated NF- κ B [78,80]. It interfered with the canonical NF- κ B signaling pathway by downregulating the expression of p65/NF- κ B, IKK γ (NEMO), and IKK β in pancreatic cancer cells, which is partly responsible for its capabilities of growth inhibition and apoptosis induction [25]. Besides influencing tumor cell proliferation and survival, NF- κ B also promotes invasion, metastasis, and angiogenesis. Many studies have revealed α -MG's inhibitory activities against invasion and migration of cancer cells, and the inhibition of NF- κ B is one of the most important mechanisms for its antimetastatic effects. For example, α -MG exhibited a significant antimetastatic effect against TPA-stimulated human breast cancer MCF-7 cells through the inhibition of nuclear translocation and DNA binding activity of NF- κ B via blocking phosphorylation and degradation of I κ B α [117]. It has been reported that functions mediated by NF- κ B are at least partially performed in cooperation with STAT3. Actually, besides inhibiting the NF- κ B pathway, α -MG exposed to pancreatic cancer cells inhibited the constitutive expression of

STAT3 proteins and its phosphorylation [80]. Shan et al. [118] observed that α -MG inhibited proliferation and promoted apoptosis of gastric adenocarcinoma BGC-823 and SGC-7901 cells, possibly by suppressing the activation of STAT3 and the expression of its regulated genes, including Bcl-xL and Mcl-1. The overview of all the aberrant molecules and signaling pathways modulated by α -MG is shown in ► **Table 2**.

Toxicity and Pharmacokinetic Studies

As mentioned above, an impressive number of studies demonstrate that α -MG possesses chemopreventive and therapeutic potential in cancer. In order to successfully translate such promising observations from the preclinical to clinical stage, studies on its toxicological profile as well as pharmacokinetics and bioavailability are mandatory. Within the past years, several research groups have evaluated the potential *in vivo* toxicity of α -MG (pure compound or as a constituent of mangosteen fruit extract) primarily in rodents. Ibrahim et al. [119] firstly performed a complete *in vivo* toxicological evaluation of α -MG. Oral gavage with α -MG at a single dose as high as 1000 mg/kg BW (acute toxicity) did not produce any toxicity in ICR mice regardless of gender, as demonstrated by no occurrence of treatment-related adverse effects on BW, organ weight, serum biochemistry, histopathology, and oxidative stress biomarkers. Jujun et al. [120] reported that the ethanol extracts of mangosteen administered orally to rats at a highest single oral dose of 5000 mg/kg BW (acute toxicity) and a highest daily dose of 1000 mg/kg BW for 28 days did not trigger any significant dose-related systemic toxicity as well as hematologic and histopathologic changes. Consistently, another study performed in mice by Bunyong et al. [121] also did not find any evidence of toxicity of mangosteen extracts at a single oral dose of 5000 mg/kg BW (acute toxicity) and daily dose of 2000 mg/kg BW for 14 days (subacute toxicity). Hutadilok-Tawatana et al. [122] demonstrated that hydroethanolic rind extracts of mangosteen administered orally to rats daily as high as 1200 mg/kg BW for 12 weeks (subchronic toxicity) did not cause any changes of general behavior and physiological status throughout the study period. After 12 weeks, no significant dose-related hematologic changes were observed among the female groups, although a dose variation increase of direct bilirubin was detected in all male groups. Moreover, neither gross necropsy nor histopathological examination of vital organs revealed any abnormalities regardless of gender. However, Gutierrez-Orozco et al. [123,124] recently reported that dietary α -MG at a dose of 112 mg/kg BW exacerbated DSS-induced injury and promoted microbial dysbiosis independent of mouse strain (C3 H, Balb/c, Nude FoxN1^{nu}, and C57BL/6J mice). It caused loss of BW, greater inflammatory and crypt injury scores, immune cell infiltration, ulceration, and an increased degree of hyperplasia and epithelial cell proliferation in the colon. Also, in a non-DSS-treated mice diet with α -MG, epithelial cell proliferation and immune cell infiltration were also detected. This dose of α -MG has been reported to be safe and effective in inhibiting or retarding the growth of implanted tumors *in vivo* without observed toxicity, as shown in other studies [21,22].

► **Table 2** Molecular mechanisms behind the anticarcinogenic activities of α -MG.

Molecular mechanisms	Models	References
Modulation of phase I and II enzymes		
Mammary tumorigenesis ↓, CYP1A1 ↓, CYP1A2 ↓, CYP1B1 ↓	<i>Ex vivo</i> mouse mammary organ culture assay	[33]
CYP19/aromatase activity ↓	Microsomal	[34]
Protein expression and activity HO-1 ↑	Cerebellar granule neurons	[35]
Antioxidant		
aSMase activity ↓	Enzyme derived-bovine brain	[56]
Making SOD, GPx, CAT recover to normal level	Isoproterenol-induced myocardial infarction	[38]
Attenuation of inflammation		
NO release ↓, PEG2 release ↓, iNOS expression ↓, COX-2 expression ↓	LPS-stimulated RAW264.7 macrophage cells	[61, 62]
Protein and mRNA expressions of COX-2 ↓, PEG2 ↓	Human breast cancer MDA-MB-231 cells	[64]
Direct interaction with COX-2 and iNOS	Molecular modeling and docking study	[65]
SIRT-1 ↑, p65/NFκB acetylation ↓, COX-2 expression ↓, iNOS expression ↓, NO production ↓, PEG2 level ↓	LPS-stimulated U937 cells	[67]
Induction of cell cycle arrest		
cyclinD1/CDK4 expression ↓, p27KIP1 expression ↑, cyclinE expression ↓, phosphorylated Rb expression ↓ direct interaction with CDK4	Human prostate cancer LNCaP cells and 22Rv1 cells Molecular modeling and docking study	[71]
G1-arrest, cyclin D1 expression ↓, p21WAF1 expression ↑	Human melanoma SK-MEL-28 cells	[72]
G1-arrest, p21CIP1 expression ↑, cyclins expression ↓, cdc(s) expression ↓, CDKs expression ↓, PCNA ↓, CHEK2 expression ↑	Human breast cancer MDA-MB-231 cells	[73]
G1-arrest, cyclin-D1 expression ↓; G0/G1-arrest	Human pancreatic cancer BxPC3 and PANC1 cells	[74, 79]
G1-arrest, p16 INK4A ↑, p38MAPK ↓, Bmi-1 ↓; G2/M arrest, topoisomerases activity ↓ Direct interaction with topoisomerases	Human colon cancer HCT116 cells Thermal transition analysis	[76, 80]
Induction of the apoptotic signaling pathway		
Caspase-3 expression ↑, mitochondrial membrane potential ↓, cytochrome c expression ↑, Akt1/NF-κB expression ↓, Akt phosphorylation ↓	Human melanoma SK-MEL-28 cells	[72]
Mitochondrial membrane potential ↓, caspase-7, -8, -9 and -3 expression ↑, ROS ↑, Bcl-2 expression ↓, Bax expression ↑, Hsp70 protein expression ↓, NF-κB translocation ↓, PARP cleavage	Human breast cancer MDAMB-231 cell	[73, 78]
Caspase-3,-9 expression ↑, mitochondrial membrane potential ↓, ROS ↑, ATP ↓, cytochrome c/AIF release	Human leukemia HL60 cell	[87]
Mitochondrial membrane potential ↓ cytochrome c release, caspase-3, -8, -9 expression ↑, Bcl-2 expression ↓, Bax expression ↑, phospho-ERK, JNK, and Akt ↓	Human chondrosarcoma SW1353 cells	[88]
Mitochondrial membrane potential ↓, cytochrome c release, caspase-3,-8,-9 expression ↑, tBid ↑, Bax expression ↑, p53 expression ↑, Bmf expression ↑, Fas expression ↑	Human colon cancer COLO 205 cells	[89]
Endonuclease-G release, microRNA-143 expression ↑, ERK5 expression ↓ phospho-Akt ↓, c-Myc ↓, phospho-Erk1/2 ↑ ↓	Human colon cancer DLD-1 cells	[90]
Bcl-2 expression ↓, Bax expression ↑, p53 expression ↑	Head and neck squamous cell carcinoma HN-22, HN-30 and HN-31 cells	[91]
Caspases-8, -9, and -7 expression ↑, cytochrome c release, PARP cleavage, Bax expression ↑, p53 expression ↑, Bcl-2 expression ↓, Bid ↓, ERα expression ↓	Human breast cancer MCF-7 cell	[94]
Inhibition of angiogenic and metastatic progression		
VEGF expression ↓	Breast cancer T47D cells	[99]
VEGF-A/SDF-1 secretion ↓	Activated pancreatic stellate cells	[101]
VEGFR2 ↓, EKR1/2 ↓	Retinal microvascular endothelial cells	[102]
Phosphorylation of VEGFR2 ↓	Human umbilical vein endothelial cells	[103]
MMP-2/MMP-9/NF-κB/Akt1 expression ↓	Human melanoma SK-MEL-28 cells	[105]
MMP-2/NF-κB expression ↓, IκBα expression ↑	Human squamous cell carcinoma A-431 cells	[106]

continued

► **Table 2** *Continued*

Molecular mechanisms	Models	References
MMP-2/MMP-9 expression/activity ↓, αvβ3 integrin ↓, FAK ↓, ERK1/2 ↓, IκBα degradation ↓, NF-κB DNA binding ↓	Human lung adenocarcinoma A549 cells	[107]
MMP-2/MMP-9/u-PA expression ↓, JNK1/2 phosphorylation ↓, NF-κB/AP-1 binding activity ↓	Human prostate carcinoma PC-3 cells	[108]
MMP-2/MMP-9/E-cadherin expression ↓, ERK phosphorylation ↓	LPS-induced human pancreatic cancer MIAPaCa-2 and BxPC-3 cells	[109]
MMP-2/MMP-9/vimentin/N-cadherin expression ↓, E-cadherin expression ↑, Akt phosphorylation ↓	Human pancreatic cancer BxPC-3 and Panc-1 cells	[101]
MMP-2/MMP-9 expression ↓, ERK1/2 ↓, IκBα degradation ↓, nuclear levels of NF-κB/AP-1 ↓, NF-κB/AP-1 DNA binding ↓	TPA-induced human breast MCF-7 cells	[117]
pNF-κB/p65Ser552 ↓, pStat3Ser727 ↓, pStat3Tyr705 ↓, MMP9 ↓, DNA binding activity of NF-κB/Stat3 ↓, TIMP1 expression ↑	Human pancreatic cancer PL-45, PANC1, BxPC3, and ASPC1 cells	[25]

Upregulation and increased activities are represented in the upper rows, and inhibition and decreased activities in the bottom rows for each model.

The absorption, transport, metabolism, and excretion of α-MG have also been investigated in several models: isolated intestinal microsomes, various human cell lines, and tissue homogenates as well as mice and rats. Using a digestion/Caco-2 human intestinal cell model, Bumrungpert et al. [125] demonstrated that (i) α-MG was taken up by Caco-2 cells in a dose-dependent manner and partially converted into phase II metabolites, and (ii) both free and conjugated forms of α-MG were transported across the basolateral membrane of Caco-2 cells, suggesting that this compound and its phase II metabolites were absorbed and bioavailable. Subsequently, α-MG was detected to be transported into various cell lines (macrophage-like THP-1, hepatic HepG2, enterocyte-like Caco-2, and colon HT-29), where it underwent phase II metabolism and biotransformation to get converted into other xanthenes [126]. By *in vitro* metabolism studies using tissue homogenates, α-MG was proven to be metabolized mainly via the liver and small intestine [127, 128]. With regards to the formation of metabolites, the glucuronidated, bis-glucuronidated, dehydrogenated, hydrogenated, oxidized, and methylated α-MG were tentatively identified [128]. *In vivo* animal studies showed that the majority of α-MG occurred in the plasma as phase II metabolites and only minimal amounts as free forms after oral administration [129]. Phase II metabolites displayed a prolonged half-life compared to the free compound because they underwent enterohepatic recirculation and were reabsorbed from the bile to the intestine and back into the bloodstream again [129]. Recently, α-MG was reported to have dose-independent (linear) pharmacokinetics at oral doses of 10–100 mg/kg BW in mice [128]. After oral administration, α-MG (10 mg/kg) was detected in the plasma at the point of 5 min, indicating a rapid gastrointestinal absorption, and was then assigned to most tissues except the brain; it was relatively high in the liver, intestine, kidney, fat, and lung [127, 128].

There has been some studies conducted to research the pharmacokinetics and safety of α-MG in animal models, albeit with confusing, conflicting, and contradictory results. Similar studies on humans are limited to date. ► **Table 3** summarizes the current knowledge on the clinical pharmacokinetic and safety studies about α-MG-containing food products. In a randomized, double blinded, placebo-controlled study, ingestion of a blended mango-

steen juice by healthy human subjects could decrease the serum CRP levels, however, other inflammation markers were increased compared to the placebo [130]. Qu and colleagues [131, 132] determined the bioavailability of the free form of α-MG and vitamins B2 and B5 found in xanthone-rich mangosteen products (Verve® and Mangosteen Plus with Essential Minerals) and its efficacy on plasma antioxidant status in the human body. The results shown that α-MG and vitamins B2 and B5 were bioavailable with an observed C_{max} at a t_{max} of around 1 h, and the antioxidant capacity measured with the oxygen radical absorbance capacity assay was increased. Additionally, Chitchumroonchokchai et al. [133] revealed the presence of free and conjugated forms of α-MG in plasma and urine after consuming a mangostin-rich mangosteen juice. In a recent randomized, double-blind, placebo-controlled clinical trial, the investigators examined the effects of a mangosteen-based beverage (Verve®) on antioxidant, anti-inflammatory, and immunity biomarkers in the plasma of healthy adults [134]. It was found that after the 30-day trial, the CRP level in the group given the mangosteen-based drink formula significantly decreased by 46%, and that antioxidant capacity in the bloodstream showed 15% more than that in the placebo group. Immunity biomarkers IgA, IgG, IgM, C3, and C4 were not affected in the mangosteen given group. Importantly, there were no side effects detected on hepatic and renal functions after long-term consumption.

The oral bioavailability of α-MG, however, is very low, only 2.29% of oral α-MG at 10 mg/kg in mice [128], which is likely due to its intensive first-pass metabolism [127, 135] as well as poor absorption caused by low water solubility and the efflux effect of P-gp [136]. The low oral bioavailability has limited its further pharmacological exploitation. There have been some preliminary studies that attempted to improve its bioavailability. For instance, Fei et al. [137] synthesized several α-MG analogs through substitution of the hydroxyl groups at C1, C3, and C6, cyclization at C-2 and C-3, and modification at C4 and at C7 with the aim of improving its antitumor activity and water solubility. Despite showing an improved water solubility, all of them reduced the activity of the parent compound. Aisha et al. [138] prepared solid dispersions of α-MG in PVP that showed improved solubility, self-assembly of nanomicelles, and intracellular delivery through endocytosis,

► **Table 3** Clinical pharmacokinetic and safety studies of α -GM containing products.

Tested product	Delivery route	Dose	Observations	References
Mangosteen supplement containing mangosteen juice, vitamins, minerals, aloe vera, and green tea	Oral	59 mL/person/day for 30 days	Decreased levels of serum CRP levels; increased ratio of T helper to cytotoxic T cells; increased serum levels of IL-1 α and IL-1 β , and complement components C3 and C4	[130]
Mangosteen beverage containing mangosteen, aloe vera, multivitamins, and green tea (Verve®)	Oral	59 mL/person (single dose)	C_{max} , 3.12 \pm 1.47 ng/mL, at t_{max} of 1 h; antioxidant capacity increased with a maximum effect of 18% after 2 h, and the increased antioxidant level lasted at least 4 h	[131]
Mangosteen beverage containing mangosteen, aloe vera, multivitamins, and green tea (Mangosteen Plus with Essential Minerals)	Oral	245 mL/person (single dose)	C_{max} , 4.16 \pm 2.85 ng/mL, at t_{max} of 1 h; antioxidant capacity increased with a maximum effect of 60% after 1 h, and the increased antioxidant level lasted at least 6 h	[132]
100% Mangosteen juice containing 58% α -GM	Oral	60 mL/person mangosteen juice with a high-fat breakfast	Free and conjugated forms were detected in serum and urine; AUC, 762–4030 nmol/L \times h, C_{max} , 113 \pm 107 nmol/L, at t_{max} of 3.7 \pm 2.4 h	[133]
Mangosteen beverage containing mangosteen, aloe vera, multivitamins, and green tea (Verve®)	Oral	245 mL/person/day for 30 days	Decreased levels of serum CRP levels; enhanced antioxidant capacity; immunity biomarkers IgA, IgG, IgM, C3 and C4 were not affected; no side effects on human hepatic and kidney functions	[134]

probably enhancing the antitumor efficacy. Zhao et al. [139] employed a soft capsule with vegetable oil as the dispersion matrix to improve the bioavailability of α -MG, and finally the absolute bioavailability was effectively improved.

Conclusion and Future Directions

The high morbidity and mortality of cancer due to the current unsatisfactory anticancer strategies has forced researchers to examine preventive approaches as well as alternative treatments. Chemoprevention by utilization of dietary phytochemicals has emerged as an economic and practicable strategy for cancer control and management. As described in the review, the data derived from various cancer cell lines as well as chemically-induced tumors and implanted tumors in animal models has shown that α -MG could effectively inhibit the process of carcinogenesis with a pleiotropic mode of action. According to the drug development pipeline, however, α -MG is still at the preclinical stage now, at which point pharmacodynamics, toxicology, and pharmacokinetic studies should be extensively performed before the drug can enter into the clinical testing phase.

The chemopreventive activities of α -MG are largely due to its inhibition of abnormal cell proliferation through the regulation of cell cycle and apoptosis as evidenced by a large number of studies discussed here. Also, the excellent antimetastatic activities have been proven in various types of cancer cells and animal models. Perhaps, future research should further characterize the exact mechanisms by which α -MG exerts its effects on the cell cycle, apoptosis, angiogenesis, and metastasis. The anticarcinogenic activities of α -MG include the mitigation of oxidative stress and inhibition of metabolic activation of carcinogens. Although α -MG is

known to scavenge ROS and modulate oxidative stress-related enzymes, it is necessary to further identify the underlying signaling pathways, thereby improving the therapeutic modalities of this compound. Besides, the studies regarding its effects on phase I and II enzymes and upstream signaling pathways associated with the modulation of carcinogen biotransformation are limited, and hence much particular attention to this area is mandatory.

Until now, only limited information with respect to the pharmacokinetic properties of α -MG is available. In particular, many questions with respect to α -MG's metabolism and excretion remain to be determined. It is still to be clarified whether α -MG conjugates are transported to tissues or directly excreted, and how biological activities will change after conjugation. Systematic pharmacokinetic studies about the establishment of an appropriate route of administration and effective concentration range under physiological conditions of α -MG have not yet been performed. Despite being well tolerated in most preclinical studies, the toxicological profile, especially chronic toxicity of ingestion, remains unclear now. This current research gap in pharmacokinetics and toxicology has hindered the clinical application of α -MG. Therefore, extensive animal studies, long-term epidemiologic studies, and controlled clinical trials are required to further establish its safety and chemopreventive efficacy. Pharmacokinetic data from studies with murine models indicates a poor bioavailability of α -MG. Another future research area would be the design of novel α -MG analogs with improved pharmacokinetic and pharmacodynamic properties. Additionally, active research should also be directed at novel drug formulation and delivery systems, such as solid dispersion and nanoparticle formulations of α -MG, which would enhance its targeting effect, bioavailability, and efficacy. Anyway, the current available preclinical and mechanistic

data has indicated that α -MG is a promising candidate to be used in cancer chemoprevention.

Supporting information

Diagrams showing the biochemical basis of α -MG as a cancer chemopreventive agent, how α -MG acts as a cancer-blocking agent obstructing cancer initiation, and how α -MG acts as a cancer-suppressing agent retarding cancer promotion and progression are available as Supporting Information.

Acknowledgements

The authors acknowledge the Leading Entrepreneurship Talents Program of Nanjing, China (No.2013B14007) for the financial support.

Conflict of Interest

The authors confirm that this article content has no conflicts of interest.

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