

Aged Garlic and Cancer: A Systematic Review

Abstract

Cancer is one of the leading causes of morbidity and mortality worldwide, which increases health-care costs. It has been reported that some dietary components such as aged garlic, one of the garlic preparations with no strong odor and harsh irritating taste, exhibits anticancer effects. This review summarizes the potential beneficial effects of aged garlic on cancer incidences as well as prevention and improvement of factors related to malignancy. Electronic databases, including MEDLINE, Web of Science, the Cochrane Library, and Google Scholar were searched. All study designs which were focused on cancer incidence, or indices related to malignancy as outcomes in human, animal, and human cells studies, and aged garlic and its ingredients as exposures were reviewed in accordance to the items for systematic reviews (PRISMA) guidelines. Initially, 304 articles were identified. Then, 25 articles which met the inclusion criteria were selected. Based on the evaluation, overall quality score of human studies was well. Although there were inconsistent evidence from human studies, results of the animal and laboratory results were mostly consistent. The overall findings may suggest that intakes of aged garlic are inversely associated with cancer. In this regard, the studies have shortcomings. Therefore, more precise investigations will be necessary to decide whether aged garlic consumption is recommendable as a part of cancer prevention or control programs. However, due to anticancer properties of aged garlic, its consumption along with healthy diet may have beneficial effects on cancer.

Keywords: Aged garlic, cancer, diet, systematic review

Introduction

Cancer is one of the leading causes of morbidity and mortality worldwide, which increases health-care costs.^[1] Multiple genetic and environmental risk factors have been mentioned for cancer.^[2] Among environmental factors, diet plays an important role. Approximately one-third of cancer cases might be prevented by dietary modification.^[3,4] There is increasing evidence that dietary patterns and dietary components such as foods and nutrients play major roles in reducing or inducing the risk of cancer.^[5-12]

Assessing the relation between the intakes of some kinds of vegetables such as allium vegetables, especially garlic and the risk of cancer is interesting. Garlic (*Allium sativum* L.) is a traditional herbal food in cancer prevention and it is mostly recommended vegetable in the pyramid by the National Cancer Institute.^[13,14]

Interest in the therapeutic efficacy of garlic has origins in antiquity. Ancient medical

texts from Egypt, Greece, Rome, China, and India each prescribed medical applications for garlic.^[15] Evidence for the anticancer effects of raw garlic also shows that it may affect cancer cells by modulation of many pathways including alteration in carcinogen-metabolizing enzymes, cell cycle arrest, and induction of apoptotic cell death and suppression of oncogenic signal transduction pathways.^[16] However, along with raw garlic benefits come some adverse effects. In addition, some people are reluctant to ingest raw garlic due to its unpleasant odor and taste.^[17] Hence, in order to reduce these attributes without losing biological functions, many types of garlic preparations have been developed.

Aged garlic is one of the garlic preparations with no strong odor and harsh irritating taste that is resulting from aging of fresh garlic in aqueous ethanol for >10 months at room temperature. During the aging processes, the levels of beneficial compounds, such as pyruvate and S-allylcysteine (SAC), are increased. Another recently identified antioxidant compound of aged garlic that is not present in raw- or heat-treated garlic

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is N-alpha-(1-deoxy-D-fructos-1-yl)-L-arginine which its activity is comparable to ascorbic acid.^[17] Some studies have also reported that the antioxidant activity of phenolic compounds of aged garlic, significantly were higher than raw garlic.^[18] Furthermore, aged garlic contains a key component called S-allylmercaptocysteine (SAMC), a water-soluble sulfur compound with antioxidant property that only appears after the aging process, inhibits cell growth, and promotes apoptosis in several cancer cell lines.^[19] In addition, it is suitable and tolerable for long-term consumption because it has rare adverse effects such as irritating.^[20] Yet, there is no report on toxic symptoms and interactions with medications of aged garlic.^[21]

Recently, there is increasing evidence of anticancer activity of aged garlic against several cancer types in human studies.^[20,22-24] Furthermore, most animal and laboratory evidence reported effectiveness of aged garlic and components derived from aged garlic on tumor markers reduction.^[25-39] However, some of them showed contradictory effects.

Based on our exploration of the scientific literature, the efficacy of aged garlic on malignancy has not been systematically evaluated. Hence, the objective of this systematic review was to organize the results of research articles which included cancer incidence or indices related to malignancy as outcome and aged garlic and its ingredients as exposures.

Methods

Search strategy

All the review process was performed according to the PRISMA criteria. We searched MEDLINE, ISI Web of Science, the Cochrane library, and Google Scholar to identify published articles up to May 2018 that evaluated the effects of aged garlic on cancer. The main search terms and format was (neoplasia) and (aged garlic). Language, publication status, type of intervention, and time restriction were not applied.

Study selection

According to our inclusion criteria, we considered human, animal, and human cell studies with cancer incidence and increase or decrease of any cancer markers such as number and size of adenomas, survival time, cell proliferation, apoptosis, and immunological indices. The significant change for each study was extracted as summary measures. Those studies that met our inclusion criteria were chosen. When title/abstract was not clear for the judgment to include the study, full text was checked. Studies were excluded if they focused exclusively on garlic and garlic extract. The process of selecting papers started with a screening of the 304 literature title. Those papers related to garlic and garlic extract (116 papers) and aged garlic without addressing cancer (147 papers) were left out. Furthermore, 15 articles were excluded because their intervention assessed animal cell line. Ultimately,

our review involved 25 studies (4 human, 6 animal, and 15 human cells studies), which met the aforementioned criteria [Figure 1]. Quality of each human study was evaluated according to Downs quality assessment and a score from 0 to 27 was given to each study.^[40] Higher scores reflected higher quality. Furthermore, SYRCLE's RoB tool, an adapted version of the Cochrane RoB tool, was used to assess animal studies methodological quality^[41] that contains 10 items to investigate sources of bias.

Data extraction

Tables 1-3 provides information includes target population, age, study design, types of aged garlic used, study duration, authors, years, country, and summary of the outcomes.

Results

Considering the type of the studies, the results were as follows:

Laboratory studies

In 15 studies, researchers examined the effects of aged garlic on the induced cancer on human cells which are summarized in Table 1. The proliferation assay showed inhibitory effect of aged garlic extract (AGE) on colorectal carcinoma cells in a dose-dependent model. Findings from the mentioned study suggest that AGE suppressed invasion of some version of cancer cells and not all of them. Suppression of invasion might be associated with enhanced adhesion and inhibited the migration endothelial cells in the presence of AGE. Furthermore, tube formation for reorganization stage of angiogenesis on endothelial cells was assayed and suggested that AGE have inhibitory effect on vessel formation at high concentration of AGE compared with low concentration.^[31] Regarding the evaluation of the Sigounas *et al.*, SAMC suppresses development and survive breast and prostate, hormone-responsive cancer cell lines. This study highlighted that the response and sensitivity are

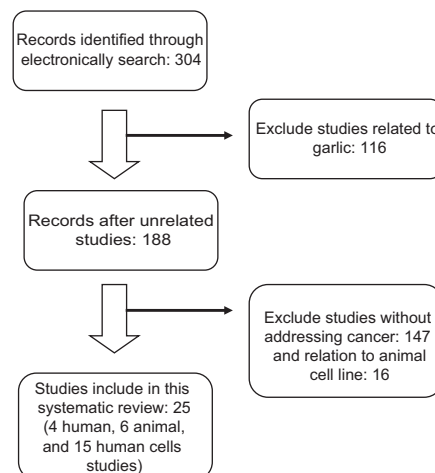


Figure 1: Study flow diagram of the search process and inclusion of studies into the systematic review

Table 1: Characteristics of various human cells studies that evaluated the effects of aged garlic on cancer

First author, journal, year of publication, country	Intervention - dose - duration	Population	Result	P
Matsuura N, J Nutr, 2006, Japan	AGE concentrations of 0.1, 1.0, and 10 g/L. 24, 48, and 72 h incubation	HT29, SW480, SW620, colorectal carcinoma cells	Suppressed the proliferation of 3 type of cells, invasive activities of SW480 and SW620, inhibiting angiogenesis	<i>P</i> <0.05
Sigounas G, Nutr Cancer, 1997, USA	SAMC (0.05 mM-1 mM)	Erythroleukemia (HEL and OCIM-1), breast (MCF-7) and prostate CRL-1740 cancer cell lines, and umbilical vein endothelial as nonmalignant cells	Antiproliferative effect on malignant cells, especially breast and prostate cells	<i>P</i> <0.05 Suppressed growth (100%) at >0.25, >0.1, and 0.05 mM SAMC for HEL, MCF-7, and CRL cells, respectively Suppressed growth (55%) at 0.1 mM SAMC for OCIM-1 cells Suppressed growth (40%) at 0.1 mM SAMC for umbilical vein endothelial compared with control
Welch C, Cancer Lett, 1992, USA	SAC (200-1600 Mg/ml)	LA-N-5 human neuroblastoma cells	Suppressed the proliferation of cells-unable to induce differentiation	<i>P</i> <0.05 Suppressed growth at >400 Mg/ml SAC
Hong YS, Exp Mol Med, 2000, Korea	DAS, DADS, garlic extract	Small cell lung cancer cells	Induced significantly apoptotic proteins in H460, H1299 cells	<i>P</i> <0.05 DAS: 35.5%±4.2%-27.7%±3.7% DADS: 37.8%±3.8%-31.4%±4.2% Garlic extract: 29.5%±3.1%-17.7±3.1%
Shirin H, Cancer Res, 2001, USA	SAMC-SAC (50 µm-250 µm) -24 and 48 h	Human colon cancer cell lines, SW-480 and HT-29	Suppressed the proliferation by SAMC (growth and cell cycle kinetics in the G2 or M Phases, induced apoptosis, increase in intracellular levels of GSH, and activation of JNK1)	<i>P</i> <0.001 <i>P</i> <0.001 <i>P</i> <0.001 <i>P</i> <0.05 <i>P</i> <0.05 (SAMC led to an 2.6-fold increase in JNK1 activity)
Chu Q, Carcinogenesis, 2006, China	SAC (100 mM) - SAMC (5 mM) different time	Prostate, ovarian, nasopharyngeal, and esophageal cancer cells	Suppression of invasive growth	<i>P</i> <0.05
Hakimzadeh H, Immunopharmacol Immunotoxicol, 2010, Iran	Garlic extract and its fractions including residue (R) at 5, 24, and 48 h	Melanoma cells	Garlic extract and R100 had cytotoxic activities against Sk-mel3 melanoma cells dilution 1:2 of garlic extract has most cytotoxic effect	<i>P</i> <0.05 In optimum conditions, cytotoxic effect of garlic extract, R100, and R10 is 92%, 92%, and 86%, respectively
Lee Y, Biol Pharm Bull, 2011, Korea	SAMC-100 and 300 mg/kg	Gastric cancer cells	Apoptotic effect of SAMC and increases in bax mRNA	31.36% (<i>P</i> <0.05) and 37.78% (<i>P</i> <0.01) by 100 and 300 mg/kg SAMC
Lee Y, Int J Mol Med, 2008, Korea	SAMC (0 µm-400 µM) -48 h	Gastric cancer cells	The antiproliferative and apoptotic effect	<i>P</i> <0.05 Viability inhibited >48% and >80% in malignant cells exposed to 200 and 400 µM of SAMC, respectively Caspase-3 activities increased 2.1-, 2.6-, and 3.1-fold by SAMC (200, 300, and 400 µM, respectively)

Contd...

Table 1: Contd...

First author, journal, year of publication, country	Intervention - dose - duration	Population	Result	P
Sun HJ, Asian Pac J Cancer Prev, 2013, China	SBC or SAC, 1, 2.5, 5, 7.5, 10, and 15 mM, 12, 24, 48, and 72 h	Gastric cancer cells	Inhibition rates of tumor survival Induced apoptosis blocked the cell cycle at G2- phases Decreased the amount of mitochondrial membrane potential Induced apoptosis through different pathway	<i>P</i> <0.05 SBC or SAC for 72 h, the inhibition rates were elevated from 5.04% to 76.66% and 2.0% to 37.68%, respectively, <i>P</i> <0.01 Increased apoptotic cells from 2.04% to 6.17% <i>P</i> <0.05 Elevated G2-phase from 8.98% to 12.66% <i>P</i> <0.05 Decreased from 97.62% to 77.09% <i>P</i> <0.05 Increased caspase-3, and caspase-9 to 201.52% and 322.73% <i>P</i> <0.05 Decreased Bcl-2 expression to 39.48%, Bax and increased p53 expression to 356.03% and 200.21%, respectively
Yan JY, Eur Rev Med Pharmacol Sci, 2013, China	SAMC, 0-400 μM, 72 h	Gastric cancer cells	Induced apoptotic morphology Decreased tumor cell viability Increased Cellular total RNA of apoptosis pathway members	<i>P</i> <0.05 <i>P</i> <0.05 <i>P</i> <0.05
Tong D, Oncol Rep., 2014, China		Hepatocellular carcinoma and colon cancer cell	Increased apoptosis rate through induced TGF-β and reduced MAPK signaling pathway	<i>P</i> <0.05
Wu J, Exp Mol Pathol., 2016, China	SAMC, C (50, 100, 150, 300, and 600 μmol/l), 8 h	Ovarian cancer cells	Inhibition of HO8910 and SKOV3 cells proliferation Induction apoptosis	<i>P</i> <0.05 <i>P</i> <0.05 <i>P</i> <0.05
Wang K, Int Immunopharmacol., 2016, China	SAMC-1, 10 or 50 μM	precancerous carcinogenesis in human lung cells	Inhibition invasion Suppression of cell proliferation	<i>P</i> <0.05 The 50 μM SAMC CoTM treatment inhibited B (a) P-induced cell proliferation at 3, 6, 12, and 24 h by an average of 87%, 112%, 173%, and 85%, respectively. The 50 μM PreTM SAMC treatment inhibited B (a) P-induced increases in cell proliferation at 3, 6, and 24 h by an average of 84%, 109%, and 144%, respectively
Kumar S, Appl Biochem Biotechnol., 2015, India	ASL50, 36 μg/ml, 48 h	Oral carcinoma KB cells	Antiproliferative activity and apoptosis of cancer cells by inducing higher caspase enzyme activity	<i>P</i> <0.05

SAMC=S-allylmercaptocysteine, AGE=Aged garlic extract, SAC=S-allylcysteine, DAS=Diallyl sulfide, DADS=Diallyl disulfide, GSH=Glutathion, JNK1=c-Jun N-terminal protein kinase, TGF-β=Transforming growth factor beta, MAPK=Mitogen-activated protein kinases

Table 2: Characteristics of various animal studies that evaluated the effects of aged garlic on cancer

First author, journal, year of publication, country	Intervention - dose - duration	Population	Result	P	Quality score (%)
Uda N, J Nutr, 2006, Japan	AGE: 2, 5, and 10 mL/kg, 6 weeks	Male rats, 5 weeks of age	Reduced the numbers and areas of GST-P-positive foci (preneoplastic lesions) reduction in BrdU	$P<0.01$ $P<0.05$ $P<0.05$	30
Katsuki, J Nutr, 2006, Japan	AGE mixed with diet at a concentration of 4%	Male rats, 5 weeks of age	Reduced the tumor incidence in the small intestine versus control, tumor multiplicity in colons, tumor multiplicity in small intestine, colonic ACF formation, MIB-5-labeling index	$P=0.0004$ (33.3% vs. 93.3%) $P=0.0081$ (1.0±1.0 vs. 3.7±1.0) $P=0.0001$ (0.4±0.7 vs. 1.6±1.3) $P<0.0001$ $P<0.0001$ (22.9±8.3 vs. 37.8±9.0)	30
Cohen La, Nutr Cancer, 1999, USA	666 and 2000 ppm SAC, 7 days	Male rats, 5 weeks of age	Not inhibitory effect on any index of mammary tumorigenesis (multiplicity, volume, latency) versus control	$P>0.05$ (2.5±2.0 vs. 1.95±1.7) $P>0.05$ (17.1±17.4 vs. 11.1±19) $P>0.05$ (95±23.7 vs. 92±27.7)	30
Shirzad H, J Med Food, 2011, Iran	AGE (3-month-old)- 20 mg/kg - 3 weeks	inbred BALB/c mice	reduced the mean tumor size	$P>0.05$	30
Ebrahimipour S, Pharmacognosy Res, 2013, Iran	AGE (100 mg/kg) 28 day	BALB/c mice, 6-8 weeks	Not effect on survival times Nonsignificantly reduced tumor volume Non significantly increased CD4+/CD8+ (cluster of differentiation) ratios Not effect on cytotoxic activity of splenocytes	$P=0.70$ $P>0.05$, 2% $P=0.62$ $P>0.05$ $P<0.05$	30
Fallah-Rostami F, N Am J Med Sci., 2013, Iran	AGE 100 mg/kg, day 28	BALB/c, 8 weeks	Increased production of IFN- γ Increase in lifespan Increased survival times (day) Increase in the level of IFN- γ and not effect on IL-4 production	$P<0.01$ $P<0.01$ $P<0.01$ in AGE=52.4±2.1 versus control group=35±1.8 $P<0.01$ $P>0.05$	30

AGE=Aged garlic extract, SAC=S-allylcysteine, GST-P=glutathione S-transferase placental, ACF=Aberrant crypt foci, MIB-5=Monoclonal antibody, IFN- γ =gamma interferon, IL-4=interleukin 4

different between cell variations.^[34] Evaluation of the relevant literature supported the inhibitory effect that SAC exerted on proliferation and differentiation of human neuroblastoma cells on time- and dose-dependent manner.^[36] Another trial which was conducted by Hong *et al.*, non-small cell lung cancer cell line with normal p53, tumor suppressor gene product, and lacking p53 gene treated with various concentration of diallyl sulfide (DAS), diallyl disulfide (DADS), and garlic extract. Analysis suggested that three treatment reduced expression of B-cell lymphoma 2 (Bcl-2) gene, and cell death modulators, in both cells. Furthermore, garlic extract had

slightly inhibitory effect in decreasing proliferation and increasing apoptosis in wild and null type cells compare with DAS and DADS.^[28] In the fifth study, culture of 2 types of human colon cancer cell with SAMC and SAC was assessed. SAMC had arrested properties in cycle cell in both the cells; however, this effect was different in SAMC concentration and type of cell. Similar results were obtained about apoptosis and mediating enzymes apoptosis such as caspase-3-like activity and c-Jun N-terminal protein kinase (JNK1) activity. However, similar report about SAC did not record. Furthermore, both SAC and SAMC confirmed increase in glutathione (GSH).^[32] Based

Table 3: Characteristics of various human studies that evaluated the effects of aged garlic on cancer

First author, journal, year of publication, country	Intervention - Population dose - duration	Population	Result	Pretreatment	After treatment	Quality score (%)	P	Statistical adjustment
Ishikava H, J Nutr, 2006, Japan	AGE (500 mg) -6 months	Patients (40) with advanced colorectal, liver, or pancreatic cancer 20 years or more	Increase NK cell count and NK cell activity - no difference in quality of life	207±142 27.26±15.9	277±150 36.0±13.2	81.4	P<0.05 P<0.01	Not reported
Tanaka S, J Nutr, 2006, Japan	AGE 2.4 mL/day as a case group AGE 0.16 mL/day as a control -12 months	Patients (51) with colorectal adenomas 40 and 79 years	Number of adenomas decreased to 0.63±0.32 and size of adenomas decreased to 0.86±1.08 mm	-	-	70.3	P=0.04 P=0.03	Not reported
You Wc, J Natl Cancer Inst, 2006, China	AGE (400 mg) 7.3 years	3365 participants (2258 seropositive - 1107 seronegative) 35-64 years	No decrease in the incidence of gastric cancer	OR: 0.98 (0.79-1.22)	OR: 1.02 (0.86-1.21)	81.4	P=0.45	Age, sex, and other treatments
Ma Ji, J Natl Cancer Inst, 2012, China	AGE (400 mg) 14.7 years	3365 participants (2258 seropositive -1107 seronegative) 35-64 years	No decrease in the incidence of gastric cancer	-	OR: 0.80 (0.53-1.20)	81.4	P=0.38	Age, sex, smoking, and alcohol

NK=Natural killer cells, AGE=Aged garlic extract, OR=Odds ratio

on Chu *et al.* study, many types of human cancer cell lines, with SAC and SAMC were treated. Although SAMC was more effective than SAC, both of them exerted a suppressing effect on colony-forming, developing, and invasion rate on prostate cancer cells. Similar about these data were demonstrated about ovarian, nasopharyngeal, and esophageal cancer cell lines.^[25] Other evidence addressed that SAMC induces apoptosis in gastric tumor cells by modulate the apoptotic protein expression. Furthermore, SAMC worked on inhibition of tumor growth in a concentration-dependent manner.^[42] Likewise, another study appeared apoptotic effect of SAMC on human gastric cancer cells in a dose-dependent manner. It was noted that this property related to handling apoptotic proteins and enzymes.^[30] Another same study emphasized SAMC effects on JNK and P38 apoptosis pathway.^[43] Furthermore, SAMC can induce the apoptosis of cancer cells by activating the transforming growth factor-beta and inhibiting mitogen-activated protein kinase signaling pathway in hepatocellular carcinoma and colon cancer cell line.^[44] Moreover, another water-soluble allyl sulfide isolated from AGE, S-benzyl-cysteine (SBC), could have a cytotoxic activity on human gastric cancer through different pathways such as inducing cell cycle arrest and apoptosis.^[45] In addition, another study demonstrated that garlic extract and its fractions provided properties against melanoma. Among garlic extract fraction, R100 fraction more effective than others in suppressing melanoma cell line.^[27] The inhibitory effect of SAMC was also investigated on three ovarian cancer cell lines, HO8910, HO8910PM, and SKOV3. SAMC effectively induced apoptosis in the HO8910 and SKOV3 ovarian cancer

cells by increasing the activity of caspase-3 and inhibited cells proliferation. In addition, invasion by reduced the expression of the adhesion factor, integrin β 1, and induced the expression of E-cadherin was suppressed. However, differences of mRNA levels of the apoptosis-related genes: Bcl-2-associated X protein (Bax), Bcl-2, caspase-3, and survivin, between the SAMC-treated ovarian cancer cell lines were not significant. Further analyses showed that overexpression of surviving, a member of the inhibitor of apoptosis family, is a factor responsible for differential responses of ovarian cancer cells to SAMC. Furthermore, the mice were inoculated subcutaneously with HO8910, SKOV3, and HO8910PM ovarian cancer cells. Then, they subjected to intragastric administration of 0.3 mg/g (body wt) of SAMC daily for 21 days. The result showed that SAMC reduced the volume of subcutaneous tumors derived from HO8910 and SKOV3 cells.^[39] Furthermore, inhibitory effects of SAMC against precancerous carcinogenesis in human lung cells (A549 cell line) were examined. A549 cells were either pretreated (PreTM) or concurrently treated (CoTM) with 1 μ M benzo (a) pyrene (B [a] P) as a carcinogen and either 10 or 50 μ M SAMC. The 50 μ M PreTM group inhibited B (a) P-induced cell proliferation by approximately 100%. The 50 μ M SAMC PreTM and CoTM inhibited the B (a) P-induced G2/M phase shift by 100% and 97%, respectively. Furthermore, the mechanisms involved in SAMC inhibition of B (a) P-induced carcinogenesis were suppression of cell proliferation, cell cycle regulation, attenuation of reactive oxygen species (ROS) formation, inhibition of DNA damage, increase of superoxide dismutase (SOD) activity, and inhibition of

nuclear factor-kappa B (NF- κ B) activity.^[38] In another study, biological properties of *Allium sativum* lectin 50 protein (ASL50) from aged *A. sativum* bulbs were investigated. ASL50 exhibited antiproliferative activity on oral carcinoma KB cells with an IC50 of 36 μ g/ml after treatment for 48 h and induced the apoptosis of cancer cells by inducing 2.5-fold higher caspase enzyme activity, a family of protease enzymes playing essential roles in programmed cell death, than untreated cells.^[37]

Animal studies

Six animal studies about cancer and aged garlic were identified and included in this review. Data extraction of these studies is shown in Table 2. In one of them, AGE contained 0.1% SAC was administered in different dose during the promotion phase of carcinogenesis. The placental GSH S-transferase (GST-P) form, new protein marker for preneoplastic liver foci, and bromodeoxyuridine (BrdU)-labeling indices, proliferation rate marker, were evaluated. The areas and numbers of GST-P-positive foci and BrdU labeling index reduction following administration of AGE in a dose-dependent manner were significantly addressed.^[35] Another study emphasized that diet containing the equivalent of 4% AGE could decrease the number of tumors in the small intestine. Moreover, the number of aberrant crypt foci, the earliest changes seen in the colon leading to cancer, and monoclonal antibody MIB-5 labeling index, the tool for determining proliferating cells, were reduced in the AGE-treated group as compared to untreated group.^[29] Results of another research suggested that different types of garlic preparations had different pharmacological properties in tumor cells in mice. These results showed although 3-month-old garlic extract could reduce tumor size, fresh garlic might cause desirable anticancer effects compared with other types of processed garlic extracts because of highest content of bioactive components.^[33] In contrast to previous studies, one study showed that component derived from aged garlic, SAC, had not actions of cancer chemoprevention in rat mammary tumor.^[26] Another study had documented that AGE had synergistic effects with naltrexone as an opioid receptor antagonist on malignancy assessment markers such as inhibition of tumor growth and increment of survival times.^[46] There is also a report regarding the growth-inhibiting activity of AGE on fibrosarcoma cells implanted in mice.^[47] Overall, most animal studies demonstrated a protective effect of AGE in the incidence or growth of carcinogen-induced tumors or tumor biomarker.

Human studies

A limited number of human cancer studies have been conducted with aged garlic. Table 3 are summarized the details of these informations. In one trial, the participants with advanced liver cancer or advanced pancreatic cancer, or advanced colon cancer were allocated into two groups, AGE group and placebo group. Participants in AGE group

consumed the daily dose of AGE twice a day. They have been followed for 12 and 24 weeks after the beginning of the trial. Although no significant changes were identified in indices of cell-mediated immunity and other markers after AGE administering compared with control group, there was particular difference in natural killer (NK) cell activity in AGE group, especially in patients with advanced cancer of the gastrointestinal system. These improvements of immunity function may help to survive. These results were based on a small sample of patients with different types of cancer. Hence, it would be better if trial were performed with just focusing on one kind of cancer. Moreover, adjustment for confounding factors such as chemotherapy, drugs, and diet was not performed in the mentioned study. In addition, patients in control group consumed garlic when they knew beneficial effects of garlic during completing consent form. Ultimately, it would be better if death due to cancer has been followed up in this investigation.^[22] Another trial showed that daily consumption of 2.4 mL AGE in 51 diagnosed patients as carrying colorectal adenomas could significantly inhibit the number and size of adenomas in the treatment group after 6 and 12 months.^[20] However, we should keep in mind that protective factors of colorectal adenoma such as folate and regular nonsteroidal anti-inflammatory drug using and risk factors such as smoking and alcohol drinking might have confounding role in the obtained results.^[48] Further to mentioned point, it also seems that assessment of reliable markers of genetic risk factors of colorectal cancer is necessary.^[49] In another trial in this field, researchers reported that supplementation with AGE had not considerably chemoprevention effects on the incidence of precancerous gastric lesions.^[24] Even longer follow-up provided the same results.^[23] The obtained result may be due to differences of geographic regions and genetic factors. In other hand, *Helicobacter pylori* infection is an independent risk factor of gastric cancer.^[50] Furthermore, the prevalence of *H. pylori*-seropositive in the county of this study was 67%. Therefore, it needs to investigate the association between aged garlic intake and incidence of gastric cancer in the countries with lower prevalence of *H. pylori*. Moreover, origin of *H. pylori* strains affects rates of gastric cancer^[51], and it may reduce the effect of interventions such as AGE supplementation. It seems necessary to evaluate the *H. pylori* strains when diet intervention is assessed. Furthermore, variability reading of the histopathology may affect the power to detect the effects of intervention.

Discussion

To the best of our knowledge, this is the first systematic review that evaluates the effects of aged garlic on cancer incidence prevention and improvement of indices related to malignancy. The human evidence in relation with aged garlic is insufficient and we identified four studies, with mentioned strategies. Although well-designed studies, long length of follow-up, treatment compliance, large number

of participants, and low number of lost participants are strengths of most of these human evidence, the lack of studies evaluating in this field may be explained by the fact that most of the patients with cancer are hospitalized and have severe side effects of cancer that it cause to limit the study duration. Furthermore, it is also possible that bias may be related to unintended exposure to aged garlic and/or its products among participants in the control group. It must be kept in mind that several confounding factors such as dietary and supplement intakes have not been taken into account in these papers. Furthermore, received anticancer treatments during the studies by patients may probably affect aged garlic intervention.

In relation to possible components that could modify the effects of aged garlic, especially in human trial, many factors might have essential roles. Based on one report, dietary habits in patients with cancer may have changed after the diagnosis of this disease. This behavior changes may influence on dietary interventions studies.^[52] Moreover, administrated forms of AGE such as garlic fluid or powder extract, garlic preparation process, daily dose, duration of the study, or active components contained in each intervention were different. In addition, cancer phases, cancer type, correct staging of cancer, and differential diagnoses are critical and these may affect the assessment of recurrences or later development of malignancy. Furthermore, there are many of garlic's variety in different geographical region which have different active substances and effects.^[53] According to critical roles and predictive values of some prognostic factors such as gene expression, tumor markers, and age factor on tumorigenesis, it seems to be important to examine simultaneously heterogeneity of gene and age with aged garlic intervention in the mentioned studies.^[54,55] Furthermore, for preventive purposes, nutrients with anticarcinogenic properties should be used an early stage of carcinogenesis. Therefore, daily, long-term, and early possible ingestion of anticancer foods are important in cancer prevention or relief symptom.^[24]

In contrast to human studies, the results of the animal and laboratory results were mostly consistent and only some of them showed contradictory results. Animal and laboratory studies had generally poor quality and their results were not in the same line with clinical trials, which limits their usefulness to human cancer treatment. Although these models have so far been fruitful in understanding the mechanism of aged garlic intervention on malignancy, these results are not applicable to humans because of their biological differences. Hence, these findings must be repeated and confirmed by human models.

Possible mechanisms of AGE action are related to its variety compounds. The major unique organosulfur compounds in aged garlic are water-soluble SAC and SAMC, which have potent antioxidant activity. The lipid-soluble organosulfur components include DAS, DADS, diallyl polysulfides, and

others compounds such as allixin demonstrate antioxidant activity.^[24] According to the reports, in malignant cells, high level of oxidative stress reaction and reactive species are essential as signal transduction for high proliferation rate. Hence, in the field of treatment of cancer, regulation of oxidative stress is novel therapeutic approaches.^[56] Therefore, oil-soluble and water-soluble compounds of aged garlic that have scavenging activity for ROS can decrease some tumor markers.^[24] Furthermore, SAC component increases antioxidant enzymes such as GST activity that causes enhancement of detoxification of carcinogens and inhibition of both the formation and bioactivities of carcinogens. SAC also decreased mutagenicity and binding of carcinogens to DNA by reduced formation of DNA adducts, a piece of DNA covalently bonded to a cancer causing.^[24] Other study indicated that induced GSH by AGE can change the level of reactive oxygen and is able to protect cells from carcinogenesis by scavenging free radicals, which may reduce cancer development.^[25] Stimulating the expression of nuclear factor – E2-related factor 2 and inhibit prooxidant enzymes such as inducible nitric oxide synthase by AGE and its components also may have potential effects on prevention oxidative stress. AGE probably exerts its inhibitory effect on oxidative stress by suppressing the influx of some metal ions through chelating them.^[57] Furthermore, the mechanisms involved in SAMC inhibition of induced carcinogenesis are attenuation of ROS formation, inhibition of DNA damage, increase of SOD activity, and inhibition of NF- κ B activity.^[38] Recently, researches have provided evidence that immune system has the great potential for the specific elimination of tumors.^[58] Moreover, AGE may be a natural and excellent adjuvant to excite the immune system and increased interleukin 1, 2, and 4 (IL-1, IL-2, and IL-4), gamma interferon cytokines, and NK cells activity to improve antitumor immunity.^[24,59] Fraction 4, a protein fraction isolated from AGE, is a very efficient immunopotentiator and enhanced the cytotoxicity through augmenting macrophage, stimulated the lymphokine (IL-2)-activated killer activity against tumor cells.^[20] AGE also can act as angiogenesis inhibitor, and it has been proposed as angiopreventive, the prevention of motility, proliferation, and tube formation of endothelial cells through the inhibition of angiogenesis and were thought to be potential as cancer inhibitor.^[33] Moreover, AGE affects regulator proteins that regulate cell death. Mechanism of apoptosis induced by AGE are regulated through p53, which regulates the cell cycle and functions as a tumor suppressor, or related to Bax/Bcl-2 or JNK1, that plays roles in the cellular apoptosis pathway.^[25,32] In addition, SAC and SAMC demonstrated potent inhibition of vascular endothelial growth and exerted suppressed effect of colony-forming, development, and invasion rate of cancer cells. Invasive phenomenon accompanies with increase mesenchymal and decrease epithelial markers, such as E-cadherin and γ -catenin. Both compounds could reduce mesenchymal markers, such as α -smooth muscle actin (α -SMA) and vimentin.^[25,31]

Our findings must be interpreted in the context of possible weaknesses. First, because of the insufficient studies included, due to limited access to some databases, and the natures of the studies, the findings cannot be used to infer causality. Second, most survey, particularly human studies were performed in particular population, and these results cannot be attributed to other populations. Although the mean score quality for all human articles considered was high (78.6%), considering the risk of bias across studies such as publication bias, performance bias, reporting bias, and potential conflicts of interest are important. Therefore, more clinical trials and prospective cohort human studies with adequate sample size in at-risk participants and patients with cancer in developed and developing countries are needed. It seems that pilot test assessment of risk of bias tools using a small subset of studies that represent the range of risk of bias in the evidence base is necessary. As mentioned previously, although SYRCLE's RoB tool was used to assess animal studies quality and all these studies achieved the same score quality (30%), but all of laboratory studies have not been reported their limitations; they have provided a clear definition of the available standard protocols used in methods.

Conclusions

Overall, the current findings are not sufficient to assess the effects of aged garlic on cancer. However, due to anticancer properties of aged garlic, its consumption along with healthy diet may have beneficial effects on cancer. More clinical trials and prospective cohort human studies with adequate sample size are necessary.

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Conflicts of interest

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