Chapter 10
An Evidence-based Perspective of *Allium Sativum* (Garlic) for Cancer Patients

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Abstract *Allium sativum* (garlic) has been used since prehistoric times in various cultures as a spice as well as a medicine to combat microbial and fungal infections, help in cardiovascular problems, stimulate immunological system or stop tumor growth. Epidemiological studies indicate that increased consumption of garlic is inversely correlated with the risk of different types of cancer in various human populations. Garlic preparations inhibit chemically induced cancers in animals. This chemo-preventive activity is attributed to organosulfur compounds which modulate Phase I and II detoxification enzymes, thus inhibit pro-carcinogen activation and/or enhance carcinogen neutralization and removal. Laboratory studies also indicate that garlic compounds suppress cancer development at post-initiation phases inducing cell cycle block and apoptosis as well as inhibiting angiogenesis and metastasis. Results of the *in vitro* studies explain the mechanisms of action of garlic organosulfurs at the molecular level, which is a necessary step before their clinical use for cancer patients. This chapter reviews the evidence on garlic chemo-preventive activities in human populations, animal models and limited clinical trials. It also summarizes the current knowledge on molecular mechanisms of its anti-proliferative activity toward cancer cells, possible interactions with drugs and impact on immune system—factors that should be considered before use of garlic compounds in cancer therapy.

10.1 Introduction

Allium sativum (garlic) is a member of the *Liliaceae* family, which also includes onions, leeks, scallions or chives. Garlic is rich in sulfur-containing compounds, which contribute to its characteristic odor, taste and beneficial health effects. Thus, it has been used as a spice as well as a medicine since prehistoric times

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W. C. S. Cho (ed.), *Evidence-based Anticancer Materia Medica,* Evidence-based Anticancer Complementary and Alternative Medicine,
DOI 10.1007/978-94-007-0526-5_10, © Springer Science+Business Media B.V. 2011

in various cultures (Rivlin 2001). Ancient Egyptians, Jews, Greeks and Romans consumed garlic to increase their strength, courage in battle and enhance work capacity. The *Codex Ebers*, an Egiptian medical text dating to 1500 BC, mentions garlic as a remedy for skin diseases, poisoning, heart problems, and abnormal growths (tumors). Hippocrates prescribed garlic for protecting the skin against toxins or treating abdominal tumors. In ancient China and Japan garlic was thought to provide energy, lift depression, and improve male potency, aid respiration and digestion. In India, 2,000 years ago, garlic was used to treat arthritis and heart disease.

In the modern era health benefits of garlic are well recognized and include antimicrobial, anti-fungal, lipid and glucose lowering, anti-thrombotic and immunostimulatory properties, as well as anticancer activity. The evidence for the chemopreventive and therapeutic application of garlic preparations is reviewed below.

10.2 Bioactive Compounds Derived from Garlic

Garlic bulbs contain approximately 65% water, 28% carbohydrates, 2.3% organosulfur compounds, 2% protein, 1.2% free amino acids and 1.5% fiber (Block 1985). The biological activity of garlic is attributed to organosulfur compounds (OSCs). The primary sulfur-containing compounds in whole and intact garlic are γ-glutamyl-*S*-alk(en)yl-*L*-cysteins. They are precursors of odorless alliin (*S*-alk(en)yl-*L*-cysteine sulfoxide). Processing of garlic bulbs (cutting, chewing or crushing) releases a vacuolar enzyme, alliinase, which acting upon alliin gives rise to allicin (diallyl thiosulphate), the principal active substance of fresh garlic extract, discovered by Cavallito in 1944 (Cavallito and Bailey 1944). Allicin is unstable and breaks down readily to produce odorous oil-soluble sulfur compounds, including diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), allyl methyl trisulfide, dithiins and ajoenes (Fig. 10.1). Water-soluble garlic sulfur compounds, such as S-allylcysteine (SAC) or S-allylmercaptocysteine (SAMC), are products of the bioconversion of γ-glutamyl-*S*-alk(en)yl-*L*-cysteins which takes place during natural aging of plants (Fig. 10.1).

The composition and thus biological activity of garlic extracts depends on the mode of preparation. For example, rehydratated standardized powder of crushed garlic is almost devoid of alliin because rehydratation activates alliinase. It contains allicin (13.5 mg/g DW), allyl sulfides (0.15 mg/g DW, including DATS- 56–87% and DADS- 9–31%), γ-glutamyl-*S*-allylcysteine (5.9 mg/g DW), γ-glutamyl-*S*-*trans*-1-propenylcysteine (5.8 mg/g DW), S-allylcysteine (0.28 mg/g DW), S-allylmercaptocysteine (< 0.02 mg/g DW) (Lawson and Wang 2005). Garlic oil obtained by steam distillation contains diallyl- (57%, including DADS and DATS), methyl allyl- (37%) and dimethyl oligosulphides (6%) (Lawson et al. 1991). On the other hand, oil macerated garlic, produced by homogenizing chopped garlic in vegetable oil, contains primarily vinyldithiins (70%), dialk(en)yl sulfides (18%) and ajoenes (12%) (Lawson et al. 1991). Aged garlic extract (AGE) which results from pro-

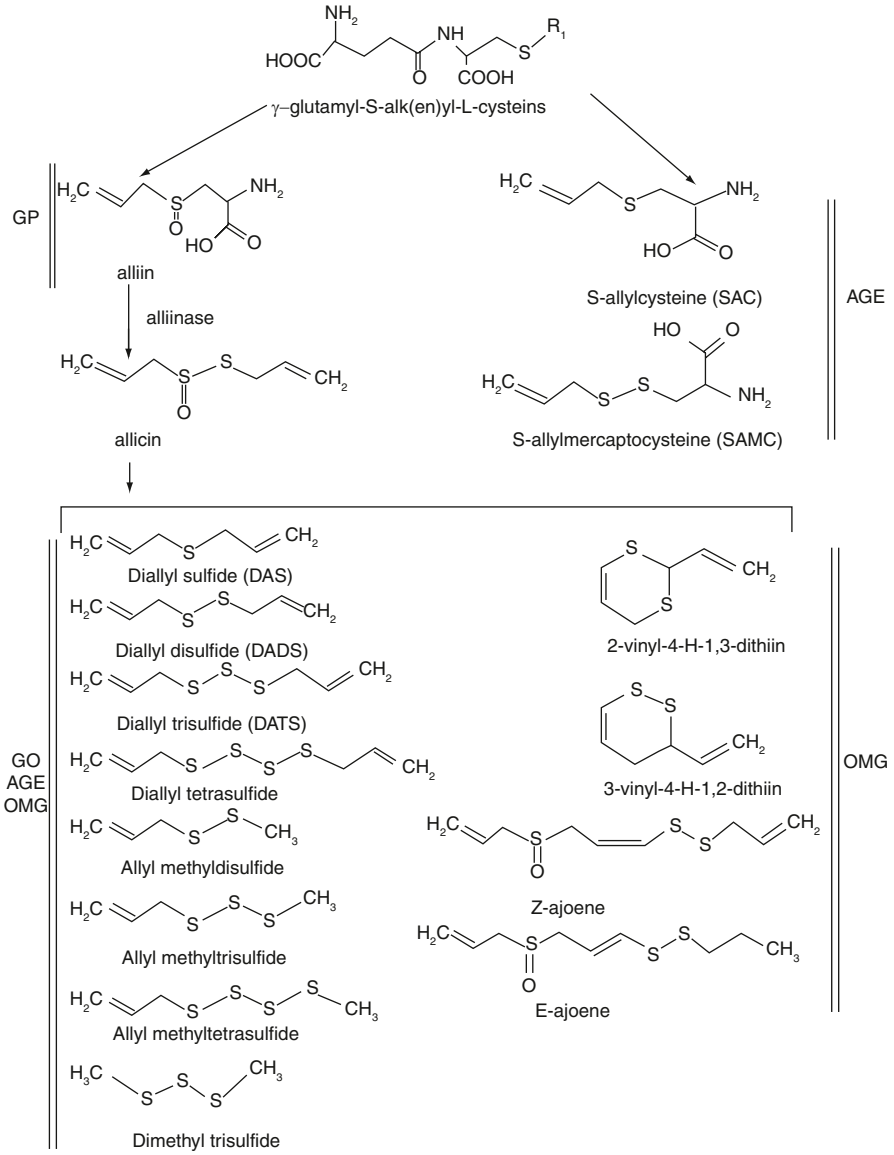

Fig. 10.1 Generation of garlic organosulfur compounds and their representation in various garlic preparations, such as: AGE: aged garlic extract, GP: garlic powder, GO: garlic oil and OMG: oil-macerated garlic. R1-allyl- or alkenyl-group

longed extraction of fresh garlic in ethanol solution at room temperature is devoid of allicin and contains water-soluble organosulfurs, such as S-allylcysteine, S-allylmercaptocysteine and lipid-soluble DAS, DADS, diallyl polysulfides and others (Weinberg et al. 1993) (Fig. 10.1).

10.3 Human Studies

Epidemiological and intervention studies were carried out to determine the association between garlic consumption and cancer risk (Table 10.1). The majority of these studies were related to addressing the chemo-preventive role of garlic consumption in regard to stomach and colon cancer. In the case of stomach cancer, most of the studies were population studies carried out in China and regarded mainly the comparison of death rates or cancer incidents in low-risk and high-risk areas for stomach cancer. The lower death rates/cancer incidences were attributed to the consumption of garlic. In the low-risk Cangshan County the consumption of garlic was reported to be 9 times higher than in the high-risk Linqu County of the Shandong Province (You et al. 1998, 1999). A 3-fold increase in garlic intake was associated with the low cancer incidence in a low-risk area in the Jiangsu Province (Takezaki et al. 1999). In another study the increased consumption of *Allium* vegetables, including garlic, was found to be inversely associated with the risk of stomach cancer (Gao et al. 1999). Although these results are promising they do not provide the necessary information regarding the validation of the food-frequency assessment, therefore according to the FDA evidence-based review system, are not statistically significant (Kim and Kwon 2009).

In a case-control study carried out in China a group of 564 patients with stomach cancer was compared to a control group of 1,131 residents from a high-risk area. The study showed that the consumption of various *Allium* vegetables, including garlic, reduced the occurrence of stomach cancer (You et al. 1989). Another case-control study performed in Korea indicated a significant decrease in the risk of gastric cancer with the increased intake of garlic (Kim et al. 2002).

In a double-blind intervention study the effects of high doses of allitridum (DATS) and low doses of selenium were examined on the occurrence of gastric cancer in the Qixia County, Shandong Province. Case subjects were selected based on criteria such as medical history of stomach disorder, family history of stomach cancer, alcohol consumption and/or smoking. 2,526 case subjects were enrolled in the trail and were given allitridum (200 mg/day) and selenium (100 μg every other day) for 1 month each year for 3 consecutive years. The control group consisted of 2,507 subjects, which were given a placebo. Five years after the termination of the study the occurrence of cancers was registered. 35 and 51 cases of malignant tumors were documented in the intervention group and control group, respectively, and the morbidity rates of all cancers in the intervention group declined by 22%. In the case of gastric cancer 10 and 19 cases were recorded in the intervention and control

Table 10.1 Studies on the evaluation of garlic intake and cancer risk

Study type	Cases/controls	Garlic preparation	Effects	References
Stomach cancer				
Case-control	564/1,131	Garlic (kg/year) 0.1–1.5 >1.5	OR 0.8 (S) OR 0.7 (S)	You et al. 1989
Case-control	338/669	Garlic, frequency of intake >2 times/month	OR 0.89 (NS)	Hansson et al. 1993
Cohort	139/3123	Garlic supplement any/day/1 year (*vs* other supplement)	RR 0.93 (NS)	Dorant et al. 1996b
Case-control	153/234	Garlic 1–3 servings/month >4 servings/month	OR 0.4 (S) OR 0.3 (S)	Gao et al. 1999
Case-control	136/136	Garlic (raw), highest quartile	OR 0.53 (S)	Kim et al. 2002
Intervention	2526/2507	Allitridum (DATS) 200 mg/1 month/year/3 yrs	RR 0.36 (S)	Li et al. 2004
Intervention	3365 cases	200 mg AGE and 1 mg steam distilled garlic oil—2/2 × day/7.3 years	RR 1.06 (NS)	You et al. 2006
Colorectal cancer				
Cohort	212/35,004	Garlic >1 servings/week	Distal colon RR 0.52 (S) Total colon RR 0.68 (NS)	Steinmetz et al. 1994
Cohort	205/47,949	Garlic >2 servings/week	Colon RR 0.77 (NS) Distal colon RR 0.63 (S)	Giovannucci et al. 1994
Case-cohort	443/3,123	Garlic supplement any/day/1 year (*vs* other supplement)	RR 0.93 (NS)	Dorant et al. 1996a
Case-control	488/488	Garlic >3 servings/week	OR 0.66 (S)	Witte et al. 1996
Case-control	1192/1192	Garlic, highest tertile	OR 0.8 (S)	Le Marchand et al. 1997
Case-control	223/491	Garlic, highest tertile	OR 0.39 (S)	Levi et al. 1999
Intervention	37 cases	AGE, 2.4 ml/day/12 months	RR 0.71 (NS)	Tanaka et al. 2004
Case-control	2280/4765	Garlic, high intake	OR 0.74 (S)	Galeone et al. 2006

Table 10.1 (continued)

Study type	Cases/controls	Garlic preparation	Effects	References
Prostate cancer				
Case-control	328/328	Garlic, frequency of intake >2 times/week	OR 0.64 (NS)	Key et al. 1997
Case-control	238/471	Garlic > 2.14 g/day	OR 0.47 (S)	Hsing et al. 2002
Case-control	1294/1451	Garlic, high intake	OR 0.81 (NS)	Galeone et al. 2006
Cohort	1338/29,361	Garlic >1 serving/week	RR 0.88 (NS)	Kirsh et al. 2007
Breast cancer				
Cohort	469/1713	Garlic supplement any/day/1 year (*vs* other supplement)	RR 0.87 (NS)	Dorant et al. 1995
Case-control	345/345	Garlic, frequency of intake		Challier et al. 1998
		7–10 times/week	OR 0.52 (S)	
		11–12 times/week	OR 0.25 (S)	
Case-control	2900/3122	Garlic, high intake	OR 0.9 (NS)	Galeone et al. 2006
Lung cancer				
Cohort	484/3,123	Garlic supplement any/day/1 year	RR 1.78 (NS)	Dorant et al. 1994
Laryngeal cancer				
Case-control	201/414	Garlic, highest tertile	OR 0.5 (NS)	Zheng et al. 1992a
Case-control	527/1297	Garlic, high intake	OR 0.56 (S)	Galeone et al. 2006
Nasal cancer				
Case-control	60/414	Garlic daily	OR 0.6 (NS)	Zheng et al. 1992b
Esophageal cancer				
Case-control	196/392	Raw/cooked (kg/year) 0.6–2.0	OR 0.6 (S)	Hu et al. 1994
		>2.0	OR 1.0 (NS)	
Case-control	395/1066	Garlic, high intake	OR 0.43 (S)	Galeone et al. 2006

NS: not significant, OR: odds ratio—highest *vs* lowest consumption, RR: relative risk, S: significant

groups, respectively, and the morbidity rate declined by 47.3% in the intervention group (Li et al. 2004).

In a latter study carried out in the Linqu County of the Shandong Province, a long-term trial was conducted to evaluate, among others, the effects of AGE and steam distilled garlic oil on the development of advanced precancerous gastric lesions. The study was a factorial-design trial, in which 3,365 case subjects were given a one-time antibiotic treatment for *Helicobacter pylori* infection and/or 7.3 years of oral vitamin (vitamin C, E and selenium) supplementation and/or garlic supplementation. The results showed no association between long-term garlic supplementation and precancerous gastric lesions (You et al. 2006). The results of a cohort study carried out in the Netherlands and a case-control study carried out in Sweden showed that intake of garlic supplements did not correlate with the risk of stomach cancer (Dorant et al. 1996b; Hansson et al. 1993).

In the United States two large cohort studies were conducted to determine the correlation between garlic intake and colorectal cancer. In the first study, The Iowa Women's Health Study, a group of 41,837 women were interviewed for their dietary habits and were then monitored for cancer incidence over a period of five years. During this time 212 cases of colon cancer were documented. The results of the study showed that the consumption of more than 1 serving of garlic per week significantly decreased the risk of colorectal cancer (Steinmetz et al. 1994). In another cohort study, 47,949 men were followed for approximately 6 years. 205 cases of colon cancer were identified during this period. The results, however, did not associate garlic intake with colon cancer (Giovannucci et al. 1994). These results were further confirmed by a case-cohort study carried out in the Netherlands. Dietary habits of 120,852 cases were evaluated with a food-item frequency questionnaire. After a 3.3-year follow-up, 443 case subjects diagnosed with colorectal cancer and 3,123 randomly selected healthy control cases were analyzed and no association between garlic intake and colon cancer risk was reported (Dorant et al. 1996a).

In a case-control study conducted in Switzerland, including 223 case patients and 491 control subjects, the correlation between garlic consumption and colorectal cancer was shown (Levi et al. 1999). These results were supported by another case-control study carried out in Italy and Switzerland. 1,394 colon cancer cases, 886 rectal cancer cases and 4,765 control subjects were examined. The specific amounts of garlic intake were not given since garlic intake was specified in terms of qualitative variables such as non-use, low, intermediate or high. It was showed that intermediate to high use of garlic is associated with the reduced risk of colorectal cancer (Galeone et al. 2006). Two other case-control studies carried out in the United States and Hawaii confirmed that garlic intake was inversely associated with the development of adenocarcinomas (Le Marchand et al. 1997; Witte et al. 1996). The results of these two studies, however, where not considered as significant, as assessed by the FDA evidence-based review system.

A double-blind, intervention trial carried out in Japan was performed to evaluate the effects of AGE on patients with colorectal adenomas. 51 patients diagnosed with colorectal cancer were divided into an intervention group (high dose AGE,

2.4 ml/day) and control group (low dose, 0.16 ml/day). The results, verified after 6 and 12 months, showed no effect of AGE on the incidence of polyps, however AGE was found to decrease the size of existing carcinomas. Due to the low number of subjects and short duration of the trial, the results can only suggest the preventative role of AGE on colorectal adenocarcinomas (Tanaka et al. 2004).

The association between garlic consumption and the reduced risk of prostate cancer was also examined. In a case-control study carried out in England, 328 men diagnosed with prostate cancer and the same number of population controls were examined regarding their dietary habits, which included the consumption of garlic. The results showed that the intake of two or more servings of garlic per week was not associated with the reduction of prostate cancer risk (Key et al. 1997). In another study carried out in Shanghai, the association between garlic consumption and reduced prostate cancer risk was much more pronounced. 238 men diagnosed with prostate cancer and 471 men, as population control subjects, were interviewed to determine the amounts of *Allium* vegetables they consumed. The results showed that men who consumed more than 10.0 g/day had a significantly lower risk of prostate cancer compared to those who consumed less than 2.2 g/day (Hsing et al. 2002). Two other studies did not report an association between garlic intake and prostate cancer risk (Galeone et al. 2006; Kirsh et al. 2007).

Garlic consumption was also found to be inversely related to the risk of breast cancer as shown in a European case-control study conducted in France. 345 women diagnosed with primary breast carcinoma and 345 healthy women were evaluated for their dietary habits. The results of the study showed that an increase in garlic intake (more than 7 servings per week) reduced the risk of breast cancer (Challier et al. 1998). A case-control study carried out in Italy and Switzerland did not report an association between garlic intake and risk of breast cancer (Galeone et al. 2006). Similarly, a cohort study carried out in the Netherlands also reported no correlation. A cohort of 469 patients with breast cancer and 1,713 control subjects were analyzed after a 3.3-year follow-up and no association of garlic intake with breast cancer risk was reported (Dorant et al. 1995). The associations between garlic consumption and other cancers are presented in Table 10.1.

On the basis of the presented studies it is clear that to some extent there is a correlation between garlic intake and some types of cancer. The published epidemiological studies suggest the protective role of garlic against colon and stomach cancer. However, these studies do have some limitations, which decrease their credibility. One of these limitations is the diverse efficacy of the different types of garlic preparations consumed by the case subjects. These studies do not provide any consistency as to the type of preparation used, therefore too many variables are introduced. Moreover, the chemical composition of the garlic preparation can be affected through the method of preparation, such as cooking, extracting, etc. Another limitation is providing the appropriate data as to the amounts of garlic consumed. These are often not precisely specified and are reported as frequency instead of amount. Therefore, in order for the results of the studies to be more consistent, clinical trials need to be carried out to provide more credible evidence as to the association between garlic consumption and cancer development.

10.4 Evidence of the Anticancer Activity of Garlic in Animal Models

The anti-carcinogenic properties of garlic have been examined in animal experimental models. Garlic in the form of various preparations, administered either intragastrically or percutaneously, has been shown to inhibit the carcinogenesis of the colon, skin, tongue, breast and liver, induced by a variety of carcinogens (Table 10.2). For example, the chemo-preventive effects of garlic on 1,2-dimethylhydrazine (DMH)-induced colon cancer were studied in rats. Garlic was fed to rats and DMH was injected subcutaneously for 20 weeks, once a week. The results of the experiments showed that garlic significantly reduced the incidence of tumor formation in the group of rats, in which garlic constituted 2.5% of their diet (Cheng et al. 1995).

Likewise, garlic-derived compounds have been studied for their chemo-preventive activities. DAS inhibited tumor formation of the esophagus, lung, skin, forestomach, colon and breast, whereas contradictory results were obtained in the case of liver carcinogenesis (Table 10.2). DADS was reported to provide protection against chemically induced carcinogenesis of the skin, colon, kidney, fore-stomach, prostate and breast. DATS inhibited forestomach-induced carcinogenesis, whereas SAC, colon-induced carcinogenesis. Contradictory results were obtained with SAC regarding *N*-methylnitrosourea (MNU)-induced breast cancer, the inconsistencies of the obtained results could be due to the differences in the administered doses of SAC (Table 10.2).

The anti-carcinogenic properties of OSCs were found to be associated with the number of sulfur atoms in their chemical structure. In a study evaluating the protective effects of OSCs on benzo[a]pyrene (BP)-induced neoplasia of the forestomach and lungs, allyl methyl trisulfide (AMT), allyl methyl disulfide (AMD), DAS and DATS inhibited forestomach carcinogenesis, whereas only DAS and AMD inhibited lung carcinogenesis. These results suggested that the number of sulfur atoms in the molecule could determine the organ sites at which protection against carcinogens takes place (Sparnins et al. 1988). The number of allyl groups has also been shown to influence the inhibitory capacities of OSCs. Studies showed that OSCs containing allyl groups are more potent inhibitors of carcinogenesis (Sparnins et al. 1986, 1988).

Apart from the chemo-preventive properties of garlic-derived OSCs, the anticancer activities of these compounds have also been determined in animal xenograft tumor models (Table 10.3). DADS was found to inhibit the growth of HCT-15 human colon tumor xenografts in nude mice. DADS was either intraperitoneally or intragastrically administered to mice, however, its efficacy was 2 times higher when injected intraperitoneally, reducing tumor volume by 69%. The efficacy of DADS was similar to that of 5-fluorouracil (5-FU), a recognized chemo-therapeutic agent used in advanced colon cancer therapy. The concurrent treatment of DADS with 5-FU reduced the toxicity of 5-FU, improving white blood cell counts and the decrease in spleen weight and elevated plasma urea (Sundaram and Milner 1996c). DADS also suppressed the growth of KPL-1 breast cancer xenografts in nude mice.

Table 10.2 Effects of garlic and its constituents on chemically-induced carcinogenesis in animal models

Garlic preparation	Organ	Animal, dose	Carcinogen	Effect on tumor	References
Garlic	Colon	Rats, diet 2.5% (4.76 g/m^2 body surface/day)	DMH	Inh	Cheng et al. 1995
	Tongue	Rats, 250 mg/kg, po	4NQO	Inh	Balasenthil et al. 2001
Garlic oil	Skin	Mice, 10% solution, pc	BaP-croton oil	Inh	Sadhana et al. 1988
Garlic extract	Skin	Mice, 5 mg DW, pc	DMBA-TPA	Inh	Nishino et al. 1989
Garlic powder	Mammary	Rats, 1–4% in diet	DMBA	Inh	Liu et al. 1992
AGE	Liver	Rats, 2.5 & 10 ml/kg, pc	DEN	Inh	Uda et al. 2006
DAS	Esophagus	Rats, 200 mg/kg, po	NMBA	Inh	Wargovich et al. 1988
	Lung	Mice, 200 mg/kg, po	NKK	Inh	Yang et al. 2001
	Liver	Rats, 250 mg/kg, po	DEN, 2-AAF	Inh	Singh et al. 2004
	Liver	Rats, 50–100 mg/kg, po	DMH	Inh	Hayes et al. 1987
	Skin	Mice, 250 µg/mouse, pc	DMBA, TPA	Inh	Singh and Shukla 1998
	Forestomach, lung	Mice, 0.02 mmol, po	BaP	Inh	Sparnins et al. 1988
	Colon	Mice, 200 mg/kg, po	DMH	Inh	Wargovich 1987
	Colon	Rats, 50–200 mg/kg, po	Combination	No	Takahashi et al. 1992
	Liver	Rats, 50–200 mg/kg, po	Combination	Increase	Takahashi et al. 1992
	Mammary	Rats, 1.8 mmol/kg, po	DMBA	Inh	Ip et al. 1992
DADS	Mammary	Rats, 200 ppm in diet	PhIP	Inh	Suzui et al. 1997
	Mammary	Rats, 1.8 mmol/kg, po	DMBA	Inh	Ip et al. 1992
	Skin	Mice, topical application 1 mg/100 µl/2 × week/25 weeks	DMBA/TPA	Inh	Dwivedi et al. 1992
	Mammary	Rats, 57 µmol/kg diet	MNU	Inh	Schaffer et al. 1996
	Colon	Rats, 200 ppm in diet	AOM	Inh	Reddy et al. 1993
	Colon	Rats, 50–200 mg/kg, po	Combination	Inh	Takahashi et al. 1992
	Kidney	Rats, 50–200 mg/kg, po	Combination	Inh	Takahashi et al. 1992
	Forestomach	Mice, 0.02 mmol, po	NDEA	Inh	Wattenberg et al. 1989
	Prostate	Rats, 150 mg/kg, po	MNU	Inh	Arunkumar et al. 2006b

Table 10.2 (continued)

Garlic preparation	Organ	Animal, dose	Carcinogen	Effect on tumor	References
DATS	Forestomach	Mice, 0.02 mmol, po	BaP	Inh	Sparnins et al. 1988
	Lung	Mice, 0.02 mmol, po	BaP	No	Sparnins et al. 1988
SAC	Colon	Mice, 50–100 mg/kg, po	DMH	Inh	Sumiyoshi and Wargovich 1990
	Mammary	Rats, 57 μmol/kg, po	MNU	Inh	Schaffer et al. 1996
	Mammary	Rats, 666 & 2,000 ppm in diet	MNU	No	Cohen et al. 1999

2-AAF: 2-acetyl-aminofluorene, AOM: azoxymethane, BaP: benzo[*a*]pyrene, DAS: diallyl sulfide, DADS: diallyl disulfide, DATS: diallyl trisulfide, DEN: diethylnitrosamine, DMBA: 7,12-dimethylbenz(*a*)anthracene, DMH: 1,2-dimethylhydrazine, DW: dry weight, Inh: inhibition, MNU: *N*-methyl-N-nitrosourea, NDEA: *N*-nitrosodiethylamin, NMBA: *N*-nitrosomethylbenzylamine, NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, No: no effect, 4NQO: 4-nitroquinoline 1-oxide, pc: percutaneously, po: per os, PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, SAC: S-allylcysteine, TPA: 12,O-tetradecanoylphorbol-13-acetate

Table 10.3 Anticancer effects of garlic constituents in mouse xenograft models

Compound	Xenograft	Dose	Effect	References
Ajoene	B16/BL6 mouse melanoma tumor	25 µg/g bw/before tumor injection, pc 5, 15, 25 µg/g bw/every 2 days/4 weeks, pc	↓ 70–90% primary tumor growth ↓ 90% lung metastases	Taylor et al. 2006
DADS	HCT-15 human colon cancer cells KPL-1 human estrogen receptor (ER)-positive breast cancer cells	1 mg/3 × week, ip 1–2 mg/3 × week/35 weeks, ip	↓ 69% tumor volume ↓ 45% tumor volume	Sundaram and Milner 1996c Nakagawa et al. 2001
DATS	PC-3 human prostate cancer cells	6 µmol/3 × week/20 days, po	↓ 3×tumor volume	Xiao et al. 2006a
DATS in nanoparticles	HepG2 human hepatoma cells	1.5 mg/kg/every 2 days/14 days, iv	↓ 45% tumor volume	Zhang et al. 2007
SAC	CWR22R human androgen-independent prostate cancer cells	1 g/kg/day/8 weeks	↓ 62.4% tumor volume	Chu et al. 2007
SAMC	CWR22R androgen-independent primary prostate cancer cells	200 mg/kg/day/23 days, po + docetaxel 7.5 mg/kg/week/23 days, po	↑ 53% docetaxel sensitization, ↑ tumor suppression, and ↓ toxicity than docetaxel alone	Howard et al. 2008
	PC-3 androgen-independent metastatic prostate cancer cells	300 mg/kg/day/28 days, po	↓ 71% tumor volume and ↓85.5% lung and adrenal metastases	Howard et al. 2007
Thiacremonone	SW620 colon cancer	5, 10, 30 mg/kg/4 weeks, po 1 mg/kg/10 days, po, + docetaxel 1 mg/kg/1 × week/4 weeks, ip	↓ 32–50% tumor volume ↓ tumor volume and ↑mouse life span	Ban et al. 2009

DADS: diallyl disulfide, DATS: diallyl trisulfide, ip: intraperitoneally, iv: intravenously, pc: percutaneously, po: per os, SAC: S-allylcysteine, SAMC: S-allylmercaptocysteine

DADS (1 or 2 mg) was injected intraperitoneally 3 times per week from the day of tumor injection. After 35 weeks a 45% reduction in primary tumor weight was observed in DADS-treated mice, which was associated with reduced cell proliferation (Nakagawa et al. 2001). DADS also suppressed the growth of *H-ras* oncogene transformed tumor xenografts in nude mice. DADS was administered orally 3 times a week from the day of tumor injection and the results showed that the growth of tumors was significantly delayed in comparison with control mice (Singh et al. 1996).

DATS inhibited the growth of PC-3 human prostate cancer xenografts in athymic mice. DATS was administered orally (6 μmoL, thrice weekly) to nude mice with implanted PC-3 cells and after 20 days of therapy the average tumor volume in DATS-treated mice was three-fold smaller than in control mice. Moreover, the treatment did not cause weight loss or any other side effects (Xiao et al. 2006a).

S-allylcysteine was reported to inhibit the growth of human prostate CWR22R cancer xenografts in mice with no detectable side-effects. The effects of SAC were associated with the inhibition of tumor proliferation and invasion, as shown by E-cadherin restoration, and increased apoptosis (Chu et al. 2007).

Thiacremonone, a novel sulfur compound isolated from garlic, was reported to inhibit the growth of SW620 human colon tumor xenografts in mice. Furthermore, the co-treatment of thiacremonone with low doses of docetaxel (1 mg/kg) increased the susceptibility of tumor cells to docetaxel, most probably through the inactivation of NF-κB. This combined treatment reduced docetaxel toxicity and greatly increased the life span of mice from 45 to 110 days. Therefore, these results indicate that thiacremonone could be used in association with other chemo-therapeutical drugs, enabling lower dose chemo-therapuetical treatment (Ban et al. 2007, 2009).

Another OSC, SAMC, was also reported to increase the efficacy of docetaxel, as shown in a prostate CWR22R cancer xenograft model. The combination was 53% more potent in inhibiting tumor growth than docetaxel alone (Howard et al. 2008).

The anti-metastatic activities of garlic compounds have also been studied *in vivo*. The effects of SAMC were examined on the growth and tumor spread of prostate cancer with the use of a fluorescent orthotopic severe combined immunodeficient (SCID) mouse model. PC-3 cells, constitutively expressing GFP, were injected into the dorsal prostate of SCID mice and after 30 days, metastatic GFP-expressing tumors in lungs and adrenals were identified. The oral administration of SAMC (300 mg/kg/d) not only inhibited the growth of primary tumors by 71% but also reduced the number of lung and adrenal metastases by 85.5% and reduced the number of circulating tumor cells by 91%. No toxic effects or organ pathology were observed in SAMC-treated mice (Howard et al. 2007). Moreover, studies have shown that 200 mg/kg SAMC protects against toxin-induced liver damage with an efficacy similar to that of N-acetylcysteine, a hepatoprotective agent (Sumioka et al. 1998). Another garlic constituent, ajoene, was also found to inhibit lung metastasis of B16/BL6 melanoma cells in mice (Taylor et al. 2006).

The effects of OSCs have also been examined in targeted therapy with the use of nanoparticles. In these studies polybutylcyanoacrylate nanoparticles, filled with DATS (DATS-PBCA-NP) were targeted against orthotopically transplanted liver

hepatocellular HepG2 cells in mice. DATS-PBCA-NP was found to suppress the proliferation and induce apoptosis in transplanted cells leading to a significant inhibition of tumor growth (Zhang et al. 2007).

The results of the *in vivo* experiments are consistent and indicate the chemopreventive, anti-proliferative and anti-metastatic properties of garlic and its constituents.

10.5 Molecular Targets of Garlic Compounds—*In Vitro* Studies

A large body of research on the chemo-preventive and anticancer activities of garlic constituents has been performed on cultured cells, the best model for the investigation on the molecular mechanism of their action and important step for identification of mechanism-based biomarkers which might be useful in future clinical trials and therapies. It appears that compounds from garlic, besides prevention of initiation and promotion of carcinogenesis, act at later phases inhibiting cancer cell proliferation and viability.

Growth suppressive activity of garlic compounds, documented for cancer cells of different origin, relies on their ability to block cell cycle progression and/or induce programmed cell death (Table 10.4). Interestingly, most garlic-derived OSCs induce accumulation of cancer cells with 4 N DNA content which indicates a block in G2, M or both phases of the cell cycle.

Progression of cells from G2 into mitosis depends on the Cdk1-cyclin B1 complex, whose activity is controlled at multiple levels, such as production and association of components, their intracellular localization and reversible phosphorylation. Dephosphorylation of Cdk1 at Thr14/Tyr15 by Cdc25 family of dual-specificity phosphatases is crucial for the activity of the complex and Cdc25s activities are under control of DNA damage checkpoint kinases Chk1 and Chk2 (Peng et al. 1997; Sanchez et al. 1997). Garlic compounds negatively influence activation of the Cdk1-cyclin B1 complex. It has been demonstrated that DADS decreased Cdk1 kinase activity within 4 h of treatment in HCT-15 colon cancer cells (Knowles and Milner 1998). It correlated with reduced complex formation between Cdk1 and cyclin B1 despite the time-dependent accumulation of cyclin B1 as well as increased inactivating phosphorylation of Cdk1 and drop in Cdc25C protein level upon DADS treatment (Knowles and Milner 1998, 2000). The same compound induced inactivating phosphorylation of Cdk1 in HL-60 human leukemia cells (Tan et al. 2004) or decreased Cdk1 kinase level in PC-3 human prostate cancer cells in a dose-dependent manner (Arunkumar et al. 2006b). Similarly, DATS induced accumulation of cyclin B1 and impaired Cdk1/cyclin B1 activity in J5 liver cancer cells (Wu et al. 2004), decreased Cdc25C and Cdk1 levels and induced Tyr15 phosphorylation of Cdk1 in H358 non-small and H460 large cell lung cancer cell lines (Xiao et al. 2009).

Table 10.4 Evidence of anti-proliferative and apoptosis inducing activities of the most commonly studied garlic compounds in cancer cell lines. Molecular targets whose statuses change upon OSCs treatment are indicated

OSCs	Model	Effect	Molecules affected	References
DAS	Human neuroblastoma	Apoptosis	Ca^{2+}, Bax:Bcl2	Karmakar et al. 2007
	Human lung	Apoptosis	Bax:Bcl2	Hong et al. 2000
	Human glioblastoma	Apoptosis	Ca^{2+}	Das et al. 2007
DADS	Human colon	G2/M arrest, apoptosis	Cdk1, cyclin B1, Cdc25C, acetylo-H3, H4, Ca^{2+}, microtubule network, ROS, JNK, Bax:Bcl2	Sundaram and Milner 1996a, b; Knowles and Milner 1998, 2000; Park et al. 2002; Druesne et al. 2004a, b; Xiao et al. 2005b; Song et al. 2009; Yang et al. 2009
	Human cervical	Apoptosis	ROS, Ca^{2+}, Bax:Bcl2	Lin et al. 2008
	Human gastric	G2/M arrest	Cdc25C	Yuan et al. 2004
	Human bladder	G2/M arrest, apoptosis	ROS	Lu et al. 2004
	Human leukemia	G2/M arrest, apoptosis	Cdk1, cyclin B1, ROS, acetylo-H3, H4	Lea et al. 1999; Kwon et al. 2002; Tan et al. 2004
	Human lung	G2/M arrest, apoptosis	ROS, Bax:Bcl2	Hong et al. 2000; Wu et al. 2005
	Human prostate	G2/M arrest, apoptosis	Cdk1, cyclin B1, acetylo-H3, H4	Arunkumar et al. 2006a; 2007
	Human breast	Apoptosis	Bax:Bcl-xL, BimEL	Nakagawa et al. 2001; Lund et al. 2005
	Human glioblastoma	Apoptosis	Ca^{2+}	Das et al. 2007
	Human neuroblastoma	Apoptosis	ROS, Bcl2, JNK Ca^{2+}, Bax:Bcl2	Filomeni et al. 2003; Aquilano et al. 2007; Karmakar et al. 2007

Table 10.4 (continued)

OSCs	Model	Effect	Molecules affected	References
DATS	Human prostate	G2/M, prometaphase arrest, apoptosis	Cdk1, cyclin B1, Cdc25C, securin, ROS, JNK, ferritin, Akt, Bid, Bcl2, Bax:Bcl2	Xiao et al. 2004, 2005a, 2006b; Herman-Antosiewicz and Singh 2005; Antosiewicz et al. 2006; Xiao and Singh 2006; Herman-Antosiewicz et al. 2007, 2010
	Human liver	G2/M arrest	Cyclin B1	Wu et al. 2004
	Human gastric	G2/M arrest, apoptosis	Bax:Bcl2	Lan and Lu 2004; Ha et al. 2005
	Human colon	M arrest, G2/M arrest apoptosis	Microtubule network, Cdc25C, ROS	Hosono et al. 2005, 2008; Busch et al. 2010
	Human bladder	Apoptosis	Akt, Bcl2	Wang et al. 2010b
	Human breast	G2/M arrest, apoptosis	Cyclin B1, Bax:Bcl2	Malki et al. 2009
	Human skin	G2/M arrest, apoptosis	ROS, Ca^{2+}	Wang et al. 2010a
	Human glioblastoma	Apoptosis	Ca^{2+}	Das et al. 2007
	Human lung	G2/M arrest, apoptosis	Cdk1, cyclin B1, Cdc25C, Bax:Bcl2, Ca^{2+}	Sakamoto et al. 1997; Xiao et al. 2009
SAMC	Human colon	G2/M or M arrest, apoptosis	Microtubule depolymerization, JNK, acetylo-H3, H4	Shirin et al. 2001; Lea et al. 2002; Xiao et al. 2003, 2005b
	Human leukemia	G2/M arrest, apoptosis	Acetylo-H3, H4	Sigounas et al. 1997; Lea et al. 2002
Ajoene	Human leukemia	G2/M arrest, apoptosis	ROS, Bcl2	Dirsch et al. 1998, 2002; Li et al. 2002a, b
	Human gastric	Apoptosis	Bax	Lee 2008
	Human skin	Apoptosis	Bcl2	Tilli et al. 2003
	Murine melanoma	Apoptosis	Caspase-3	Ledezma et al. 2004

DAS: diallyl sulfide, DADS: diallyl disulfide, DATS: diallyl trisulfide, ROS: reactive oxygen species, SAMC: S-allylmercaptocysteine

The mechanism of OSC-induced G2/M arrest has been well elucidated for DATS in a prostate cancer cell line model (Herman-Antosiewicz and Singh 2005; Antosiewicz et al. 2006; Herman-Antosiewicz et al. 2007, 2010; Xiao et al. 2005a, 2006b). The DATS-induced Tyr-15 phosphorylation of Cdk1 and inhibition of Cdk1/cyclin B1 activity in PC-3 cells was accompanied by down-regulation of total Cdc25C protein level an increased inhibitory phosphorylation of Cdc25C (Ser216), which was mediated by checkpoint kinase-1 (Chk1) (Herman-Antosiewicz and Singh 2005; Xiao et al. 2005a). Activating phosphorylations of Chk1 (Ser317) and other kinases engaged in DNA damage checkpoint such as Chk2 (Thr68) and ATM (Ser1981) as well as histone H2A.X, whose phosphorylation at Ser139 serves as an indicator of double strand break, points toward the genotoxic activity of DATS (Herman-Antosiewicz and Singh 2005). Whether DATS directly reacts with DNA or its effect is indirect through generation of reactive oxygen species needs to be elucidated. Antioxidants such as N-acetylcysteine (NAC) or EUK134 protected against the decrease in Cdc25C level and cell cycle arrest induced by DATS (Antosiewicz et al. 2006; Xiao et al. 2005a). Genotoxic activity of DADS was also reported by Aquilano et al. (2007) in SH-SY5Y human neuroblastoma cells and it was counteracted by over-expression of neuronal nitric oxide synthase.

It was also documented that DATS induced prometaphase arrest with characteristic changes in the tubulin network, chromatin condensation and increased Ser10 phosphorylation of histone H3 (Herman-Antosiewicz and Singh 2005) as well as accumulated anaphase promoting complex (APC) substrates (securin and cyclin B1) and hyperphosphorylated APC components (Herman-Antosiewicz et al. 2007). Interestingly, Chk1 and its upstream kinase, ATR, appeared to be mediators of DATS-induced mitotic arrest (Herman-Antosiewicz and Singh 2005; Herman-Antosiewicz et al. 2007).

Mitotic block or apoptosis induced by garlic OSCs might be the result of a disruption of the microtubule network in cancer cells by these compounds. For example, treatment of SW480 human colon cancer cells or NIH 3T3 mouse fibroblasts with 150 μmol/L SAMC caused rapid microtubule depolymerization and cytoskeleton disruption in interphase cells and perturbed spindle formation in mitotic cells. SAMC (300 and 1,000 μmol/L) induced microtubule depolymerization and inhibited *de novo* tubulin polymerization *in vitro* presumably because of direct interaction with sulfhydryl groups on tubulin (Xiao et al. 2003, 2005b). DADS (112 or 280 μmol/L) arrested SW480 in both G2 and M phase of the cell cycle. It was also shown that DADS at high concentrations (560 and 1,120 μmol/L) caused a 30% inhibition of *de novo* tubulin polymerization but did not induce depolymerization of polymerized tubulin *in vitro* (Xiao et al. 2005b). Interphase NIH 3T3 cells treated with 56 μmol/L DADS revealed disorganized microtubule cytoskeleton network. Interestingly, although mitotic spindles looked normal, "lagging" chromosomes were observed in DADS-treated cells, which were suggested to cause mitotic block and appearance of multinucleated cells (Xiao et al. 2005b). DATS, but not DADS, has been shown to induce mitotic arrest in HCT-15 and DLD-1 human colon cancer cells and it was associated with the disruption of the microtubule network in interphase cells and inhibition of spindle formation in mitotic cells (Hosono et al. 2005).

This study revealed that DATS-treated β tubulins were oxidatively modified at residues Cys12 and Cys354 to form S-allylmercaptocysteine (Hosono et al. 2005). Another oil soluble garlic compound, Z-ajoene, caused G2/M cell cycle arrest and dose-dependent reduction in microtubule network of PtK2 normal marsupial kidney cells and inhibited tubulin polymerization *in vitro* (Li et al. 2002a).

OSCs may affect cancer cell proliferation through modification of the histone acetylation level, which has an impact on gene expression. It has been reported that DADS and more potently its metabolite, allyl mercaptan, increased acetylation of H4 and H3 histones in DS19 cells and K562 human leukemic, rat hepatoma and human breast cancer cells and it was suggested that histone acetylation mediates the differentiation process of erythroleukemia cells (Lea et al. 1999). Growth inhibitory effects of allicin, SAMC and SAC on DS19 cells and SAMC on Caco-2 human colon and T47D human breast cancer cells have been correlated with increased histone acetylation, although its mechanism remains to be characterized (Lea et al. 2002). DADS-induced accumulation of Caco-2 and HT-29 colon tumor cells in G2/M phase of the cell cycle was also associated with the inhibition of histone deacetylase, hyperacetylation of H3 and H4 histones and up-regulation of mRNA and protein levels of p21, an inhibitor of cyclin-dependent kinases engaged in the progression of G1 and G2 phases of the cell cycle (Druesne et al. 2004a, b). The question as of the crucial role of p21 in DADS-induced cell cycle arrest awaits further investigation. The increase in the p21 protein level has been observed in DATS-treated PC-3 cells, however, silencing of *p21* expression through specific antisense RNA had no effect on DATS-induced G2/M cell cycle arrest (Xiao et al. 2005a).

Garlic-derived OSCs have been reported to induce programmed cell death of cancer cells and a majority of them activated the so called intrinsic (mitochondrial) apoptotic pathway, which is characterized by a loss of mitochondrial membrane potential, release of pro-apoptotic molecules, activation of caspase-9 and -3 and is regulated by the Bcl-2 family members. Numerous studies showed that treatment with garlic compounds decreases the ratio of anti-apoptotic (e.g. Bcl-2, Bcl-x_L) to pro-apoptotic (e.g. Bax, Bak, Bid) Bcl-2 family members (Table 10.4). For example, in DAS or DADS-treated H460 and H1299 lung cancer cells as well as SH-SY5Y neuroblastoma cells the ratio of Bax/Bcl-2 was increased in comparison to control cells (Hong et al. 2000; Karmakar et al. 2007). A time-dependent up-regulation of Bax protein levels with concomitant down-regulation of Bcl-x_L protein levels has been reported for DADS-treated MDA-MB-231 breast cancer cells (Nakagawa et al. 2001). Z-ajoene-induced apoptosis of HL-60 cells was associated with caspase-mediated cleavage of Bcl-2, which was inhibited by the antioxidant, NAC (Li et al. 2002b). Another study revealed an increase in production of peroxide in ajoene-treated HL-60 cells and NAC partially prevented ajoene-induced ROS generation as well as apoptosis (Dirsch et al. 1998). DATS-induced cell death of PC-3 and DU-145 prostate cancer cells, which was caused by a decrease in Bcl-2 level and its JNK-mediated hyperphosphorylation. This resulted in reduced Bcl-2:Bax interaction and activation of mitochondrial pathway of apoptosis (Xiao et al. 2004). Moreover, DATS-induced JNK activation and apoptosis were inhibited by over-expression of catalase which implies the involvement of hydrogen peroxide in apoptosis induction (Xiao et al. 2004). Other members of the Bcl-2 family have been shown to regulate OSC-induced apoptosis

as well. DATS, reducing Akt activity in PC-3 and DU145 cells and consequently the phosphorylation of its substrate Bad, caused translocation of Bad to mitochondria which contributed to the activation of the intrinsic cell death pathway (Xiao and Singh 2006). DADS or garlic extract induced JNK-dependent phosphorylation of pro-apoptotic BimEL that resulted in its translocation to the mitochondria and apoptosis induction in MDA-MB-435 breast cancer cells (Lund et al. 2005).

Oxidative stress is a common phenomenon contributing to apoptosis induction by OSCs in cancer cells. For instance, DADS induced hydrogen peroxide formation and apoptosis in HL-60 leukemia (Kwon et al. 2002) and T-24 bladder cancer cells (Lu et al. 2004). DADS-induced ROS formation in SH-SY5Y neuroblastoma cells was evident as early as 15 minutes after its administration and it was followed by oxidation of cellular lipids and proteins (Filomeni et al. 2003). It was associated with JNK pathway activation. Over-expression of Cu, Zn superoxide dismutase or pretreatment with the spin trapping molecule 5,5'-dimethyl-1-pyrroline *N*-oxide protected against DADS-induced ROS generation, oxidative damage of cellular macromolecules and apoptosis. Moreover, cell permeable JNK inhibitor I protected against DADS-induced apoptosis but not G2/M cell cycle arrest (Filomeni et al. 2003). JNK1 pathway has also been activated and played a role in apoptosis induction by the water-soluble garlic compound, SAMC in SW480 and HT-29 colon cancer cells (Shirin et al. 2001; Xiao et al. 2003).

An increase in intracellular free calcium concentration is another response of cancer cells to garlic OSCs (Table 10.4). Park et al. reported biphasic DADS-induced elevation in Ca^{2+}: rapid with peak value at 3 minutes and slow and sustained elevation till 3 hours after the addition of DADS (Park et al. 2002). It was followed by an increase in hydrogen peroxide level and caspase-3 activation. Interestingly, although NAC or catalase treatment prevented the accumulation of H_2O_2 and apoptosis, they had no effect on Ca^{2+} elevation. On the other hand, cellular calcium chelator abolished the DADS-induced elevation of intracellular calcium, H_2O_2 levels and caspase-3 activation which strongly suggested that changes in calcium homeostasis might be the earliest events in DADS-induced cytotoxicity (Park et al. 2002). It has been shown that both DAS and DADS cause an increase in Ca^{2+} in SH-SY5Y cells which led to activation of calpains, the non-caspase cysteine proteases, which contribute to cell death by the induction of the mitochondrial pathway of apoptosis (Karmakar et al. 2007). DAS, DADS, and DATS induced apoptosis of human glioblastoma T98G and U87MG cells which was accompanied by an increase in cytosolic free Ca^{2+}, expression of calreticulin and activation of caspase-4 which indicates involvement of endoplasmic reticulum stress (Das et al. 2007).

Summarizing, garlic OSCs are able to increase ROS formation and/or modify cellular proteins (S-thiolation). They induce oxidative and genotoxic stress and abnormalities in cytoskeleton function in cancer cells which lead to G2 and M checkpoint activation, disruption of calcium homeostasis and induction of the mitochondrial pathway of apoptosis.

In vitro studies demonstrate anti-angiogenic and anti-metastasis properties of garlic preparations. For instance, AGE reduced invasive potential and motility of human and rat endothelial cells by 30% in matrigel chemo-invasion assays as well as capillary-like tube formation in 3-dimensional collagen matrix assays (Matsuura et al.

2006). DATS decreased capillary-like tube formation and migration of human umbilical vein endothelial cells and at the molecular level it was correlated with Akt inactivation and reduced level of VEGF receptor 2 and secretion of VEGF (Xiao et al. 2006b). Also DADS and DAS inhibited endothelial cell proliferation and migration reducing matrix metalloproteinases MMP-2 and MMP-9 (Thejass and Kuttan 2007a, b). Water soluble SAC inhibited MDA-MB-231 breast cancer cell motility and invasion, which was accompanied by increased expression of E-cadherin and reduced MMP-2 level and activity (Gapter et al. 2008).

10.6 Antioxidant and Pro-oxidant Activity of Garlic and OSCs

There are several reports demonstrating that garlic exerts direct or indirect antioxidant activity (Imai et al. 1994). Indirect activity is related to the ability of garlic to stimulate the activity of glutathione peroxidase (GPx), glutathione reductase and superoxide dismutase in different experimental models. Moreover, AGE, SAC and SAMC exhibited direct radical scavenging activity. In addition, it was found that AGE had the capacity to chelate copper ions and inhibit copper dependent lipid peroxidation (Dillon et al. 2003). Dietary supplementation with AGE for 14 days reduced plasma and urine concentrations of 8-iso-prostaglandin F (2 alpha) by 29% and 37% in non-smokers and by 35% and 48% in smokers (Dillon et al. 2002). There are several other published data demonstrating antioxidant activity of garlic and garlic preparations, however, as mentioned below, garlic can exert some adverse effects, which could be related to oxidative stress. There is an increasing amount of evidence that depending on the experimental model, the concentration of the compounds and other possible factors, garlic preparations act either as antioxidants or pro-oxidants. Filomeni et al. (2003) demonstrated that DADS increased ROS formation in cancer cells. In addition, DATS-induced prostate cancer cell death has been related to increased ROS formation (Xiao et al. 2004). Protective effects of over-expressed catalase or low molecular weight antioxidants against DATS-induced cell death support the role of ROS in cancer cell death. The dual role of antioxidant compounds exhibiting pro-oxidant activity is quite puzzling. In the case of the pro-oxidant activity of garlic our knowledge still is not complete. Interestingly, it was observed that in genetically modified prostate cancer cells, in a way that they could not activate c-jun kinases (JNK), DATS acted as an antioxidant. These data suggested that DATS-induced ROS formation is JNK-dependent. In fact, it was observed that JNK activation led to ferritin degradation (Antosiewicz et al. 2006). Ferritin is a multisubunit protein, whose main function is sequestering iron. Ferritin degradation leads to liberation of iron which in reactions with hydrogen peroxide, lipid hydroperoxides and other compounds can generate several radical species including hydroxyl radical. DATS-induced ferritin degradation was accompanied by an increase in labile iron pool (LIP). An iron chelator completely abolished DATS-induced ROS formation which suggested that it is an iron-dependent process (Antosiewicz et al. 2006).

The exact mechanism of JNK-stimulated ferritin degradation is not known. Interestingly, overproduction of ROS leads to the activation of JNK and there is some evidence that DATS-induced JNK activation is ROS-dependent. These data suggest that DATS, and possible other OSCs, in addition to iron-dependent ROS, stimulate formation of other free radical species. On the other hand it was shown that DADS treatment increased the ferritin H protein levels in the liver (Thomas et al. 2002). Altogether, these data indicate that OSCs might act as pro-oxidants by increasing ferritin degradation and LIP or as antioxidants when ferritin levels are increased.

10.7 Adverse Effects of Garlic and Its Interactions with Drugs

Considering the fact that garlic has been consumed by humans for centuries, it is generally accepted to be a safe food. However, few studies reported on some toxic effects of garlic. Some of these effects are possibly related to its pro-oxidant activity. Gastrointestinal irritation may occur after the consumption of raw garlic, fresh garlic juice, dehydrated raw garlic powder or even after the intake of garlic tablets (Hoshino et al. 2001; Borrelli et al. 2007). Clinical studies have shown that garlic at therapeutic doses may cause mild gastric mucosa damage and around 6% of patients complained of nausea and 0.8% of bloating (Borrelli et al. 2007). Experiments performed on animals also showed several adverse effects of garlic. Rats that were fed with high amounts of raw garlic, DATS or DADS developed anaemia and demonstrated lysis of red blood cells (Munday et al. 2003). Garlic extract on one hand has been shown to reduce the clastogenic effect of some toxins in mice, on the other hand at higher concentrations it induced a clastogenic effect (Das et al. 1996). Studies performed on dogs showed that raw garlic powder caused severe damage, including erosion of gastric mucosa. Dehydrated, boiled garlic powder also caused reddening of the mucosa, whereas AGE did not cause any undesirable effects (Hoshino et al. 2001). In rats raw garlic juice at a dose of 5 ml/kg resulted in the death of some animals due to stomach injury after 21 days (Nakagawa et al. 1980). The surviving rats exhibited swelling of the liver, hypertrophy of the spleen and adrenal glands, and the decrease of erythrocytes and various morphological changes were clearly observed after 3 and 8 days in the group administered with high doses of raw garlic juice. Such changes were not observed at any time with the use of extracted-aged garlic juice (Nakagawa et al. 1980). Similar observations were done on animals treated with aqueous garlic extract (200 g/L drinking water) for 10 days, where liver injury and local inflammatory response was observed (Joseph et al. 1989). AGE has been reported to protect against heat-induced inhibition of spermatogenesis in rats whereas the administration of garlic powder (50 mg/day) led to the inhibition of spermatogenesis in rats and decreased Leydig cell function (Dixit and Joshi 1982; Hammami et al. 2008).

Despite several studies demonstrating adverse effects of garlic it can be concluded that it is a relatively safe food component. In most animal studies a very

high dose of garlic was applied, which is not normally consumed. In addition, it can be assumed that consumption of garlic with other food might reduce eventual toxic effect of the former one.

The biological effects of garlic are also related to the xenobiotic metabolism. The process of detoxification of pro-cancerogens consists of two phases. Phase I, catalyzed by the isoforms of P450 cytochromes, relies on reactions of hydroxylation, oxidation, hydrolysis, demethylation and some others yielding modified xenobiotics. Unfortunately, some products of Phase I reactions could be more toxic than parent compounds. Reactions catalyzed by Phase II detoxification enzymes promote the elimination of drugs and carcinogens from the body. Therefore, the activity of Phase II enzymes such as glutation S-transferase (GST), epoxide hydrolase, quinone oxidoreductase (NQO1) and glucuronate transferase may prevent cancerogenesis by increasing the clearance rate of toxic chemicals. The main goal of both phases of xenobiotic metabolism is to increase polarity, solubility and their subsequent excretion.

In the case of Phase II enzymes the data are consistent and clearly show that garlic as well as garlic derived OSCs increase their activity. For example, Chen et al. (2004) reported that the gene expression of NQO1 and heme oxygenase increased upon treatment with DADS or DATS. Many reports showed that OSCs increased GST activity and that allyl derivatives are more potent inducers of GST than propyl-containing OSCs (Hu et al. 1997).

The effect of OSCs on Phase I enzymes is controversial. For example, Davenport and Wargovich demonstrated that in rats, gastric intubation with a single dose of 200 mg/kg DAS, DADS or allyl methyl sulfide (AMS) decreased hepatic P450–2E1 protein by 45, 25, and 47%, respectively. On the other hand, DAS and AMS increased hepatic P450–1A2 protein levels by 282 and 70%, and DAS increased P450–1A1 protein levels by 684% (Davenport and Wargovich 2005). 1,2-dimethylhydrazine is a carcinogen activated by P450–2E1, therefore inhibition of its activity by OSCs seems to be the main mechanism of chemo-prevention (Wargovich 1987). Interestingly, it was observed that mice lacking expression of *CYP1A1, CYP1B1, CYP1A2,* and *CYP2E1* do not differ from wild-type mice. However, the *CYP* null mice have altered responses to the toxic and carcinogenic effects of chemicals as compared with wild-type mice (Gonzalez and Kimura 2003). Therefore, it can be speculated that increased activity of some P450 isoenzymes induced by OSCs could elevate toxicity of some xenobiotics but on the other hand can be also protective. Induction of Phase I and II enzymes by garlic may increase clearness of some chemotherapeutics. For example, garlic lowered the serum level of saquinavir, which is an anti-HIV drug, by as much as 50% (Piscitelli et al. 2002).

10.8 Other Therapeutic Applications

The therapeutic use of antitumor drugs has been limited by their toxicity. Treated patients often experience side effects such as nausea, vomiting, diarrhea, stomatitis, gastrointestinal ulceration and mucositis. For example, methotrexate (MTX), is an anticancer drug that has been shown to be highly effective against various kinds

of cancers, however, it is highly toxic to the rapidly dividing cells of intestinal crypts. Interestingly, weight loss in rats treated with MTX and severity of jejunal damage was reduced by provision of AGE. Moreover, experiments performed on intestinal epithelial, IEC-6 cells showed that AGE almost completely suppressed MTX-induced cell death (Horie et al. 2001). These data suggest that AGE could be a supplement of choice for patients experiencing adverse effects of chemotherapy.

The mechanism of anticancer activity of garlic is far from being completely understood, however, potentiating the immune system could be a very important one. Decline in immune function is observed in advanced cancer patients and AGE has been shown to prevent the decline of NK cell number and activity in these patients (Ishikawa et al. 2006). Furthermore, AGE stimulated immune functions, such as proliferation of lymphocyte, cytokine release, NK activity and phagocytosis. Moreover, a protein fraction from garlic has been demonstrated to enhance the cytotoxicity of human peripheral blood lymphocytes against M14 melanoma cells (Morioka et al. 1993).

10.9 Summary and Conclusions

Inhibition of cancer development is a remarkable property of garlic and its constituents documented in a large body of research showing that OSCs suppress each stage of the carcinogenesis process (Fig. 10.2). The most convincing data come from

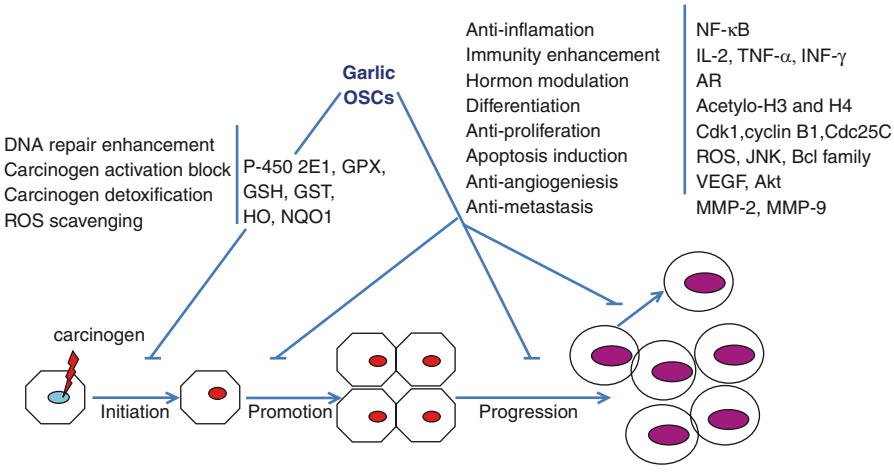

Fig. 10.2 Processes modulated by garlic and garlic-derived OSCs and their molecular targets in relation to multistage cancer development. AR: androgen receptor, Cdk1: cyclin dependent kinase 1, Cdc25C: cell division cycle 25C phosphatase, GPX: glutathione peroxidase, GSH: glutathione, GST: glutathione transferase, HO: heme oxygenase, IL-2: interleukin 2, INF-γ: interferon γ, JNK: c-jun kinase, MMP: metalloproteinase, NF-κB: nuclear factor κ B, NQO1: quinone oxidoreductase 1, ROS: reactive oxygen species, TNF-α: tumor necrosis factor α, VEGF: vascular endothelial growth factor

pre-clinical studies which demonstrate preventive activity of OSCs in chemically induced cancers in animal models as well as the ability of garlic constituents to inhibit cancer cell division, survival and metastasis both *in vitro* (cell cultures) and *in vivo* (xenograft models). However, epidemiological data and intervention trials in humans are still scarce or lacking. Thus, in order to use garlic constituents/preparations for cancer patients, additional efforts are required to: (1) assess clinical potential of OSCs in carefully designed clinical trials; (2) determine plasma and tissue concentration of OSCs in humans to relate it to effective anticancer concentrations used *in vitro*; (3) evaluate toxic amount of OSCs and their side effects on normal tissue.

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