Chapter 11
Garlic: Allyl Sulfur Compounds and Cancer Prevention

Sumeyra Cetinkaya and Ipek Süntar

Abstract Garlic, *Allium sativum* L., is a plant within the family Alliaceae that has been widely used for its culinary and medicinal properties. This plant contains organosulfur compounds with allyl groups such as allyl mercaptan (AM), S-allyl cysteine (SAC), diallyl trisulfide (DATS) and has been responsible for different health benefits such as antihypertensive, anticoagulant, anti-inflammatory, antimicrobial and anticancer. Especially some lipid-soluble allyl sulfur compounds can inactivate carcinogens and reduce cancer risk and regulate the cellular processes. Epidemiological studies have shown that garlic and its components can decrease the incidence of human stomach, colon, prostate, brain, skin, breast, lung, uterine, and esophagus cancers. These anticarcinogenic effects appear to be achieved by modifying common signaling pathways. But allyl sulfur compounds have different effect in supressing tumor proliferation. Therefore, the compounds that are responsible for the cellular and molecular effects, the stages which they suppress neoplasia and interactions with other drugs should be very well known. Tumor supression ability of allyl sulfur compounds of garlic is attributed the stimulation of detoxification enzymes, protection from oxidative stress, induction of cell apoptosis and cell cycle arrest, prevention of chromosomal damage, induction of immune system and supression of nitrosamine bioactivation. On the other hand, not only the genetic mechanisms, but also the epigenetic mechanisms can be associated with the cancer prevention. Garlic and its several allyl sulfur compounds can be modified by both DNA methylation and histon acetylation. In this chapter, preclinical and clinical studies on the effects of garlic consumption in reducing cancer prevalence will be presented in detail. Furthermore, studies involving the use of allyl sulfur compounds individually or in combination will be discussed and their mechanisms of action will be interpreted at cellular and molecular level.

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Abbreviations

AGE	Aged garlic extract
AM	Allyl mercaptan
AMD	Allyl methyl disulfide
AMPK/TSC2	AMP-activated protein kinase/tuberous sclerosis complex
AMS	Allyl methyl sulfide
Bak	Bcl-2 homologous antagonist killer
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma-extra large
Bip	Binding immunoglobulin protein
BP	Benzo[a]pyrene
Cdc2	Cell division cycle-2
Cdc25	Cell division cycle-25
Cdk	Cyclin dependent kinases
CHOP	CCAAT-enhancer-binding protein homologous protein
DADS	Diallyl disulfide
DAS	Diallyl sulfide
DATS	Diallyl trisulfide
DATTS	Dialyl tetrasulfide
DMBA	7,12-Dimethylbenz[a]anthracene
DMH	1,2-Dimethylhydrazine
DNA	Deoxyribonucleic acid
DNMTi	DNA methyltransferase inhibitors
DNMTs	DNA methyltransferases
eIF2α	Eukaryotic translation initiation factor 2α
EMT	Epithelial-mesenchymal transition
ER	Endoplasmic retikulum
ERK1/2	Extracellular signal–regulated kinases1/2
FAK	Focal adhesion kinase
FOXM1	Forkhead box protein M1
GADD153	G1 arrest and DNA damage 153
GPx	Glutathione peroxidase
GRP78	Glucose-regulated protein78
GSH	Glutathione
GSK-3β	Glycogen synthase kinase 3β

GST	Glutathione-S-transferase
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulfide
HCC	Hepatocellular carcinoma
HDAC	Histone deacetylase
HER2	Human epidermal growth factor receptor2
H-RAS	Harvey rat sarcoma viral oncogene homolog
IFNγ	Interferon-gamma
IL10	Interleukin 10
IL12	Interleukin 12
IL1α	Interleukin 1α
IL1β	Interleukin 1β
IL2	Interleukin 2
IL6	Interleukin 6
IL8	Interleukin 8
JNK	c-Jun terminal kinase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MEK	MAPK/ERK kinase
MMP	Matrix metallopeptidases
NF-κB	Nuclear factor kappa light chain enhancer of activated B cells
NO	Nitric oxide
NQO	NAD(P)H:quinone acceptor oxidoreductase
Nrf2	Nuclear factor erythroid 2-related factor 2
OSCs	Organosulfur compounds
P13k/Akt/mTOR	Phosphoinositide-3-kinase/protein kinase B
p38 MAPK	p38 mitogen-activated protein kinases
PCNA	Proliferation cell nuclear antigen
PUMA	p53 upregulated modulator of apoptosis
ROS	Reactive oxygen species
SAC	S-allyl cysteine
SAMC	S-allylmercaptocysteine
Slug (SNAI2)	Snail family transcriptional repressor 2
SOD	Superoxide dismutase
TGF-β	Transforming growth factor beta 1
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor-α
TPA	12-O-tetradecanoylphorbol-13-acetate
UGT	UDP-glucuronosyl transferase
UPR	Unfolded protein response
VEGF	Vascular endothelial growth factor

1 Introduction

Garlic *(Allium sativum* L.*)* is among the most widely used plants in the Alliaceae family, which has more than 850 different species (Sharifi-Rad et al. 2016). Bulbs of garlic are used as food and spice. Apart from its use as food, it has been used for centuries to treat various diseases such as gastrointestinal (Nicastro et al. 2015) and cardiovascular (Bradley et al. 2016) system disorders, diabetes (Bayan et al. 2014), Alzheimer's disease (Borek 2006) and for wound healing (Srimuzipo et al. 2009). In addition, previous bioactivity studies have reported that garlic displays hepatoprotective (Ajayi et al. 2009), antihypertensive (Ried et al. 2008), antihelmentic (Worku et al. 2009), antimicrobial (Yin et al. 2003), antifungal (Kutawa et al. 2018), immune modulation (Kyo et al. 2001) and anticancer (Ejaz et al. 2003) effects. Epidemiological studies have shown that garlic consumption reduce the risk of disease development. This also supports the ethnobotanical use of the plant.

Phytochemical studies on garlic have shown that various types of chemical compounds are present especially in the bulbs of this plant, including a high water content (approximately 65%). Carbohydrates (28%) (mainly fructans), proteins (2%) (mainly alliin), amino acids (1.2%) (mainly arginine), fibers (1.5%), sulfur compounds (2.3%), trace elements and phenols (Butt et al. 2009) were reported (Fig. 11.1). According to USDA database, 63.535 search results are available on garlic containing food. The main ingredients mentioned above are in different

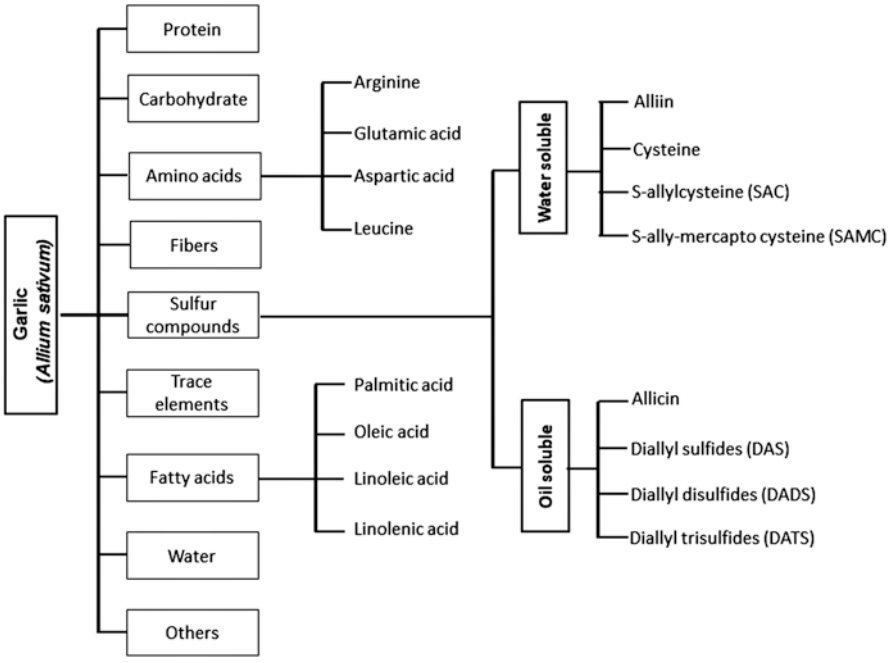

Fig. 11.1 Schematic representation of classification of the main bioactive constituents in garlic

amount for each type of food (USDA, https://fdc.nal.usda.gov). Several biological effects of garlic from wound-healing properties to anticancer effect are mainly attributed to allyl sulfur compounds and flavonoids (Putnik et al. 2018).

In vivo and *in vitro* preclinical studies on tumorigenesis have showed that garlic and its components are effective against human colorectal (Zhang et al. 2018), skin (Wang et al. 2010), prostate (Chu et al. 2006a), brain (Das et al. 2007), gastric (Ling et al. 2006), nasopharyngeal (Zhang et al. 2006), stomach (Fleischauer et al. 2000), lung (Li et al. 2012), breast (Kaschula et al. 2016), liver (Chu et al. 2013) and thyroid (Shin et al. 2010) cancers. Various mechanisms such as stimulation of detoxification enzymes, cells protection from oxidative stress, induction of cell apoptosis and cell cycle arrest, enhancement of immune system and epigenetic mechanisms are attributed to the anticancer activities of allyl sulfur compounds (Lea et al. 1999; Bruck et al. 2005; Melino et al. 2011; Upadhyay 2017). Understanding the mechanisms through which these compounds exert their biological activities is particularly important for the development of anticancer agents. It should be well known which compound or compounds are responsible for the cellular and molecular effects.

2 Organosulphur Compounds (OSCs)

The characteristic aroma of garlic is due to sulfur-containing volatile compounds that compose 1% of its dry weight (Fenwick and Hanley 1985). These volatile compounds are produced from their non-volatile precursors namely γ-glutamyl-S-alk(en)yl-L-cysteines and S-alk(en)yl-L-cysteine sulfoxides (Butt et al. 2009). OSCs are generally divided into two groups as oil-soluble and water-soluble OSCs. Unharmed cells of garlic bulbs contain alliin (S-allylcysteine sulfoxide). When the garlic is crushed, chopped or chewed, thiosulfinates whose general formula is R_1-S(O)-S-R_2 (where R_1 and R_2 are methyl, allyl, 1-propenyl) are formed (Zalepugin et al. 2015). The half-life of thiosulfinates is about 5 min in the blood (Okada et al. 2005). It triggers biochemical transformations by reacting with thiols in the cells which are in the blood or plasma. It is thought that these transformations and additions of thiol functional groups to the proteins may be related to anticancer activity (Bhuiyan et al. 2015). With crushing, chopping or chewing of the fresh garlic an enzyme known as alliinase is released and converts alliin to allicin (diallyl thiosulfinate) which is a well-known thiosulfinate. Allicin is the main chemical compound of these family, however it is stability depends on its concentration, the temperature, and the solvent in the surrounding environment (Okada et al. 2005). It is rapidly metabolized in aqueous solutions into mono-, di- and trisulfides or other organosulfide compounds such as ajoene and vinilditins (Lanzotti 2006).

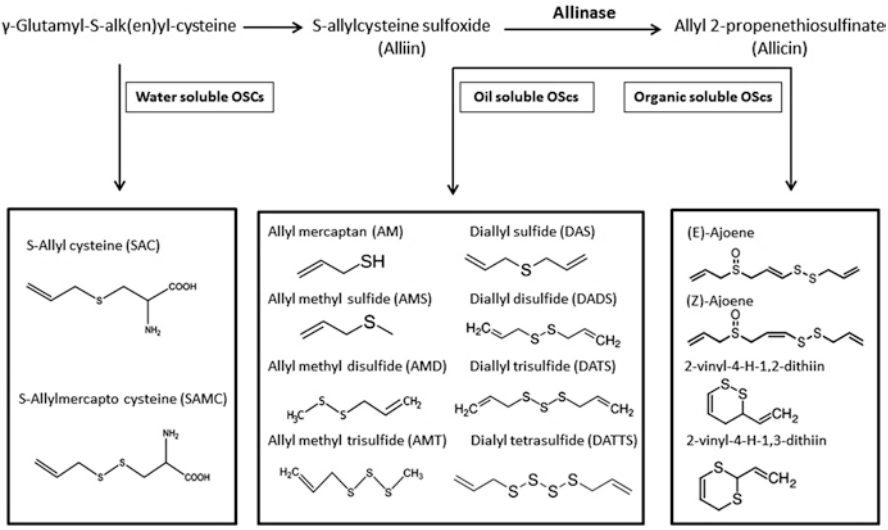

Fig. 11.2 General classification and chemical structures of commonly studied organosulfur compounds of garlic

2.1 Water-Soluble Organosulphur Compounds: S-Allyl Cysteine (SAC), S-Allylmercaptocysteine (SAMC)

γ-Glutamyl-S-alk(en)yl-cysteine is converted to SAC through γ-glutamyl transferase during the long-term incubation of crushed garlic in aqueous solutions named as aged garlic extract (AGE) (Block 1985; Jiang et al. 2019) (Fig. 11.2). One of the major bioactive components in AGE is SAC. SAC is a stable compound that prevents cardiovascular (Chuah et al. 2007), neurodegenerative diseases (Ray et al. 2011), diabetes mellitus (Sathibabu Uddandrao et al. 2017) and cancer (Ho et al. 2018). In previous studies, it was reported that SAC acts as an antiproliferative agent against some cancer types in both *in vitro* and *in vivo* models (Tang et al. 2010). It has been revealed that another water-soluble organosulfur compound, SAMC, stimulates apoptosis in breast and gastric cancer cells (Sigounas et al. 1997; Yan et al. 2013), inhibits ROS formation and DNA damage in lung cancer cells (Wang et al. 2016), changes the expression of prostate biomarkers in prostate cancer cells (Pinto et al. 2000), activates JNK1 pathway and microtubule depolymerization in colon cancer cells (Xiao et al. 2003). Moreover, both SAC and SAMC were demonstrated to inhibit vascular endothelial cell growth and suppress the effect of colony-forming, development, and invasion rate of cancer cells (Chu et al. 2006b). In addition, SAC has a 30-fold lower toxicity than allicin and DADS (Amagase et al. 2001).

2.2 Oil-Soluble Organosulphur Compounds

Steam distillation of garlic produces an oil with different allyl sulfur components such as dialyl tetrasulfide (DATTS), dialyl trisulfide (DATS), dialyl disulfide (DADS), dialyl sulfur (DAS), allyl methyl trisulfide (AMT), allyl methyl disulfide (AMD), allyl methyl sulfide (AMS) and allyl mercaptan (AM). The oil obtained by maceration contains vinilditins such as 2-vinyl-4-H-1,2-dithiin and 2-vinyl-4-H-1,3-dithiin and ajoens such as E-Ajoene and Z-Ajoene (Yoo et al. 2014) (Fig. 11.2).

The allyl sulfur compounds, which are more commonly studied in anticancer studies, are DAS, DADS, DATS and DATTS. Therefore, in this chapter we will focus specifically on anticancer activities of these compounds.

3 Cancer Chemopreventive Effects of Organosulfur Compounds of Garlic

3.1 In Vitro *Studies*

Carcinogenesis, also called oncogenesis or tumorigenesis, consists of three different stages: initiation, promotion, and progression involving invasion and metastasis. In this process, cancer hallmarks including cell proliferation, inhibition of apoptosis, invasion and metastasis, angiogenesis, immortalization, inflammation, immunity, genome instability and mutation, cell energetics and metabolism are involved (Hanahan and Weinberg 2011). Therefore, agents with therapeutic effect focus on targeting these mechanisms.

The first study to suggest that garlic can prevent the growth of malignant cells belongs to Weisberger and Pensky (1958). Table 1 presents the results of biological activity of various allyl sulfur compounds on human cell lines. Allicin, one of the most studied compounds in cancer research, induce apoptosis and interfere with cell growth signaling pathways (Lawson et al. 1992; Rose et al. 2019). However, some studies have showed that the treatment of alliin alone does not exert an antiproliferative effect on the growth of tumor cells. Therefore, it has been considered that the alliin should be broken down for maximum tumor inhibition (Scharfenberg et al. 1990).

It has been reported that SAC and SAMC regulate the expression of E-cadherin and decrease the expression of Snail, E-cadherin suppressor, in prostate, ovarian, nasopharyngeal and esophageal cancer cells (Chu et al. 2006b). E-cadherin is a transmembrane protein that plays a role in cell adhesion and is an important factor for epithelial-mesenchymal transition (EMT). Decreased level of its expression is associated with an invasive phenotype. SAC and SAMC are shown as potential agents in suppressing invasive growth (Chu et al. 2006b). Ng et al. (2012) reported that SAC significantly suppresses the expression of proliferation markers Ki-67 and

proliferation cell nuclear antigen (PCNA) and apoptosis-related B-cell lymphoma-extra large (Bcl-xL) and B-cell lymphoma 2 (Bcl-2), as well as stimulates the cell cycle arrest at S phase by decreasing cell division cycle-25 (Cdc25), cell division cycle-2 (Cdc2) and cyclin B1 expressions in the hepatocellular carcinoma cell line. They confirmed that SAC increases the level of E-cadherin and decreases the level of VEGF, similar to the studies of Chu et al. (2006b). In addition, SAC mediates the suppression of motility and invasion by stimulating E-cadherin and downregulation of MMP-2 in the breast cancer cell line, MDAMB231 (Gapter et al. 2008). According to a detailed study, DATS inhibits metastasis by inhibition of focal adhesion kinase (FAK), extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 in colon cancer cell line (Lai et al. 2015).

It was previously mentioned that all allyl sulfur compounds obtained from garlic are not equally effective in reducing tumor proliferation (Dion et al. 1997; Sakamoto et al. 1997). Similarly, fat-soluble DATS has been shown to be more effective in suppressing cell growth than DAS and DADS in HCT-5 and DLD-1 (colorectal cancer cell lines) and A549 (lung cancer cell lines) (Seki et al. 2008; Wang et al. 2014). Furthermore, DATS treatment has been reported to reduce the activity of Wnt/β-catenin by stimulating apoptosis in colorectal cancer stem cells (Zhang et al. 2018). A study on proliferation and cell cycle progression by Shirin et al. (2001) showed that SAMC, but not SAC, stops the cell cycle in the G2/M phase and activates caspase-3, triggering apoptosis in colon cancer cell lines. In addition, coadministration SAC with sulindac sulfide (SS), a chemotherapy agent in colon cancer, apoptotic and growth-inhibiting effect increased. DADS has been reported to inhibit metastasis through the SRC/RAS/ERK signaling pathway by increasing the expression of miR-34a in the breast cancer cell line, MDM-MB-23 (Xiao et al. 2014). DATS inhibits gastric cancer cell growth by regulating the expression of MMP-9 and E-cadherin proteins in BALB/c(nu/nu) mice (Jiang et al. 2017). A recent study has shown that SAC reduces viability of MCF-7 cells by decreasing the 3-mercaptopyruvate sulfur transferase (MPST) expression, H_2S/sulfane sulfur endogenous formation from L-cysteine, and sulfate sulfur level (Bronowicka-Adamska et al. 2020).

Garlic allylsulfide compounds also play a role in the cytotoxicity of cancer cells through ER stress. Ajoene, increases the level of GRP78 (Glucose-Regulated Protein 78 kDa) protein by activating the unfolded protein response (UPR) in the MDA-MB-231 breast cancer cell line and WHCO1 esophageal-cancer cells (Kaschula et al. 2016). In this way, it triggers ER stress by causing unfolded protein aggregates. A study on the colon cancer cell line showed that DATTS activates eIF2α and Nrf2/HO-1, one of the signal molecules associated with ER stress (Saidu et al. 2013). In the human malignant neuroblastoma cell line, SH-SY5Y, DAS and DADS have been demonstrated to stimulate $Ca^{(2+)}$-dependent protease calpain (Karmakar et al. 2007). Wang et al. (2012b) showed that DATS increased intracellular Ca^{2+} mobilization and expression of ER stress sensors GRP78/Bip and CHOP (CCAAT-enhancer-binding protein homologous protein)/GADD153 (G1 arrest and DNA damage 153). These studies have revealed that allyl sulfur compounds have an anticancer effect through ER stress.

In addition, oil-soluble allyl sulfur compounds in garlic are possibly more toxic than water-soluble compounds. Indeed, studies have shown that oil-soluble allyl sulfur compounds including DADS reduce the growth of neoplasms, while a water-soluble compound SAC has no effect on established tumors (Sundaram and Milner 1993; Hong 2004). Detailed information about the possible activities of allyl sulfur compounds in various human cancer cell lines is presented in Table 11.1.

Table 11.1 Selected studies that show the anticancer effects of various organosulfur compounds of garlic on human cancer cell lines

Cancer type	Effective compounds	Cancer cell type	Mechanisms of action	References
Colon cancer	Allicin (3–6μg/mL)	LoVo	Supression of adhesion, migration and invasion	Gao et al. (2009)
	SAMC (~160–175μM)	SW-480, HT-29	Inhibition of cell growth, G2/M cell cycle arrest	Shirin et al. (2001)
	SAMC (300μM)	SW480	Apoptosis via JNK1 and caspase-3 signaling pathways	Xiao et al. (2003)
	SAMC (150μM) DADS (56μM)	SW480	Microtubule depolymerization by arresting cells in mitosis	Xiao et al. (2005)
	DAS (50μM)	Colo201, Colo320, Colo320	Inhibition of N-acetyltransferase activity	Chung et al. (2004)
	DATS (40μM)	SW480, DLD-1	Supression of cell proliferation, Wnt/β-catenin pathway inhibition	Zhang et al. (2018)
	DADS (200μM)	Caco-2, HT-29	Inhibition of cell proliferation trough epigenetic mechanism; inhibition of HDAC activity, histone hyperacetylation and upregulation of p21	Druesne et al. (2004b)
	DATS (11μM)	HT-29	Inhibition of migration and invasion through MMP-2,-7,-9 and VEGF downregulation	Hosono et al. (2008)
	DATS (25μM)	HT-29, HUVEC	Inhibition of migration and angiogenesis via FAK, Src and Ras	Lai et al. (2015)

(continued)

Table 11.1 (continued)

Cancer type	Effective compounds	Cancer cell type	Mechanisms of action	References
Skin cancer	Ajoene (50μM)	B16F10 (murine)	Inhibition of tumor formation and cell proliferation	Ledezma et al. (2004)
	SAC (2.7–4.6 mM)	UCLASO-M7, M10, M12, M14, M16, M24, M25, M210, M223 (human), F10, BL6 (mouse)	Supression of cell proliferation and clonogenicity	Takeyama et al. (1993)
	DATS (50 mM)	A375	Increases intracellular ROS generation, activation of p53 pathway	Wang et al. (2010)
Prostate cancer	SAC (10–15 mM)	PC-3	Inhibition of cell proliferation, cell cycle arrest at the G0/G1 phase and induction of apoptosis through downregulation of Bcl-2 and upregulation of Bax, caspase 8	Liu et al. (2012)
	DADS (40μM)	PC-3	Induction of apoptosis and histone hyperacetylation	Arunkumar et al. (2007)
	DATS (10–40μM)	LNCaP, LNCaP-C81, LNCaP-C4-2	ROS generation, mitochondria-mediated apoptosis; upregulation of Bak and, downregulation of Bcl-2 and Bcl-xL protein levels	Kim et al. (2007)
	SAC (2.16 ± 0.32 mM), SAMC (86.34 ± 6.25μM)	PC-3	Supression of invasive growth of cancer cells through regulation of E-cadherin	Chu et al. (2006a, b)
	SAC (4.59 ± 0.93 mM), SAMC (145.79 ± 16.18μM)	DU145		

(continued)

Table 11.1 (continued)

Cancer type	Effective compounds	Cancer cell type	Mechanisms of action	References
Brain tumor	SAC (600μg/mL)	LA-N-5	Inhibition of cell growth	Welch et al. (1992)
	DAS, DADS (100μM)	T98G, U87MG	ROS production, apoptosis correlated with intracellular Ca^{+2} promotion, activation of JNK1 pathway	Das et al. (2007)
Nasopharyngeal carcinoma	SAC (10–40 mM)	HNE1, HONE1	Inhibition of invasion and migration; downregulation of FAK, Slug (SNAI2) and MMP2/9	Cho et al. (2015)
	DADS (50–150μM)	CNE-2	Cell cycle arrest at the S phase, increase of MAPK phosphorylation	Zhang et al. (2006)
Gastric cancer	DADS (30 mg/L)	MGC803	Cell cycle arrest at the G2/M phase, an alteration of the ERK1/2 signaling pathway	Ling et al. (2006)
Lung cancer	Allicin (1–20μM)	A549, H1299	Supression of adhesion, invasion and migration through decreasing the activity of the PI3K/AKT signaling pathway	Huang et al. 2017
	DADS (25–200μM)	A549	Oxidative stress mediated cell cycle arrest at G2/M and apoptosis	Wu et al. (2005)
	DATS (25–100μM)	A549	Stimulation of apoptosis through upregulation of Bax/Bcl-2 ratio and caspase-3, -8, and -9	Li et al. (2012)
	DATS (20–40μM)	LNCaP, HCT-116	Cell cycle arrest via induction of cyclin B1 and down-regulation of CDK	Xiao et al. (2009)

(continued)

Table 11.1 (continued)

Cancer type	Effective compounds	Cancer cell type	Mechanisms of action	References
Breast cancer	Ajoene (20–60μM)	MDA-MB-231	ER stress-mediated cell death via activation of UPR and upregulation of GRP78 protein	Kaschula et al. (2016)
	DATS (2.5–160μM)	MDA-MB-231, HS 578t	Inhibition of metastasis through downregulation of MMP2/9 activity by increasing the NF-κB pathway	Liu et al. (2015a), Anwar et al. (2018)
	DADS (200μM)	MCF-7	Inhibition of ERK and activation of the SAPK/JNK and p38 pathways	Lei et al. (2008)
	DADS (100–400μM)	MCF-7	Inhibition of invasion and metastasis; downregulation of vimentin, MMP9 and upregulation of E-cadherin	Chen et al. (2016)
Liver cancer	Allicin (15–50μM)	HepG2	p53 protein expression mediated apoptosis	Chu et al. (2013)
	SAC (5–50 mM)	MHCC97L	Inhibition of cell proliferation by downregulation of Ki-67 and PCNA. Inhibition of cell invasion and migration with upregulation of E-cadherin and downregulation of VEGF	Ng et al. (2012)
	SAC (0.1–100 mM)	HepG2	Stimulation of apoptosis related with caspase-8, upregulation of p38 MAPK signalling	Sengupta et al. (2017)
	DATS (10–100μM)	J5	Cell cycle arrest through accumulation of cyclin B1 and down-regulation of CDK	Wu et al. (2004)
Thyroid cancer	DAS (50–400μM)	ARO	Inhibition of cell growth and apoptosis with increase in the level of Bax, activation of caspase-9 and -3	Shin et al. (2010)
	SAMC (0.02–0.1 mg/mL)	HPACC-8305C	Apoptotic cell death and inhibit telomerase activity	Liu et al. (2015b)

3.2 In Vivo *Studies*

Effective evidence has been obtained in animal models that allyl sulfur compounds can inhibit the tumor formation as well as cancer cell growth. The intraperitoneal (i.p.) application of raw garlic extract (RGE) completely improved the mice implanted with the murine sarcoma cancer cell S180 (100 mg of the RGE for 21 days), but the same findings could not be obtained in oral application (Li et al. 2018a). Although a meta-analysis of 18 studies found a negative association between garlic consumption and reduced risk of gastric cancer (OR = 0.51, 95% CI = 0.44–0.57), prospective study results were not significant (OR = 0.95, 95% CI = 0.66–1.24) (Li et al. 2018b). These results have shown that garlic extract should not pass through the gastrointestinal tract. Garlic treatment in mice with bladder cancer has been shown to inhibit tumor growth and reduce mortality (Rigs et al. 1997). It has been reported that allicin improved liver damage and increased chemotherapy response in tamoxifen-induced mice by i.p. injection at a dose of 45 mg/kg for 7 days (Suddek 2014). On the other hand, SAMC treatment inhibited hepatocarcinogenesis by targeting the LRP6/Wnt pathway in hepatocellular carcinoma (HCC) nude mice model by daily oral gastric lavage feeding at a dose of 300 mg/kg SAMC (Xiao et al. 2018).

Administration of DAS by orally at a dose of 200 mg/kg for 7 days with Se-methylselenocysteine or quercetin to animals with 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast tumor has been shown to have a greater antitumor effect than alone treatment (Ip and Ganther 1991). DAS also inhibits tumor formation in mice and rats with benzo[a]pyrene (BP) and 1,2-dimethylhydrazine (DMH)-induced colon tumors as well as inhibits lung tumor formation in mice by reducing the metabolic activation of nitrosamine. According to these results it can be said that DAS has shown antitumor effect by affecting defective signaling pathways of various types of cancer. Similarly, DADS has been reported to decrease the NF-κB phosphorylation in azoxymethane and dextran sulphate sodium (DSS)-induced mice (60 mg/kg for 5 weeks) and prevent colitis-induced colon cancer by inhibiting GSK-3β (Saud et al. 2016). However, when DADS was given orally to H-*ras* oncogene transformed tumors in mice at a dose of 33μmol for three times per week, H-RAS mutant cancer cells growth decreased (Singh et al. 1996). In addition, when U2OS cells were subcutaneously injected to BALB/c nude mice and then treated 100 mg/kg DADS with miR-134 inhibitor for 35 days, DADS was shown to suppress forkhead box protein M1 (FOXM1)-mediated proliferation and invasion by upregulating miR-134 in osteosarcoma (Li et al. 2018c).

According to the researchers, allyl sulfur compounds reduce or suppress the growth, proliferation, invasion and metastasis of cancer when administered alone. But little is known about its clinical implications, and it should be supported by epidemiological studies. First of all, the molecular mechanism studies, including the application of both alone and combination with chemotherapy drugs, should be investigated in detail in the prevention of metastasis, which is known as the primary cause of cancer deaths.

Lai et al. (2015) treated BALB/c (nu/nu) mice with 50 mg/kg DATS for 32 days after subcutaneously injected HT-29 cells. As a result of this study, DATS was found to reduced tumor growth, tumor weight, and angiogenesis. Ajoene significantly reduced the incidence of tumors in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-promoted mouse skin, treated with 250μg ajoene for 18 weeks (Nishikawa et al. 2002). In a similar study ajoene has been shown to reduce LPS-induced TNF-α and IL6 stimulation and inhibit lung metastasis with B16/BL6 melanoma tumor model in C57BL/6 mice by i.p. injection at a dose of 25μg/g for 28 days (Taylor et al. 2006). Another study indicated that it exerts antimetastatic effect by the suppression of viable circulating tumor cells (Howard et al. 2007). In addition, DATS (6μmol orally, thrice weekly) significantly reduced tumor growth without any side effects in PC-3 xenografts in athymic mice, as well as these results correlated with the increased expressions of Bax and Bak (Xiao et al. 2006a).

In DMBA-stimulated mouse skin tumor DAS (applied topically 10 mg/kg body-weight for 24 h) suppressed the growth of tumor cells by decreasing the expression of p21/Ras oncoprotein and H-RAS mRNA level (Arora et al. 2005). Similarly, it was reported that tumor size and number decreased and p53wt and p21/Waf1 were upregulated in mice where liposomized DAS formulation (250μg, three times a week for 12 weeks) was applied against DMBA-induced skin papilloma (Khan et al. 2007). In addition, DAS (applied topically 10 mg/kg body-weight for 48 h) provided effective protection against DNA strain fractures in the DMBA-stimulated skin tumor model (Nigam and Shukla 2007). In an *in vivo* study in androgen-independent prostate cancer xenografts, oral SMAC administration (300 mg/kg for 28 days) reduced the growth of primary tumors and the number of metastases to the lung and adrenal gland (Howard et al. 2007). In the study conducted by Ng et al. (2012), it was determined that treating SAC alone or in combination with cisplatin (1 mg SAC/kg/day + 1 mg cisplatin/kg/day for 6 weeks) in the *in vivo* xenograft liver tumor model suppressed the progression and metastasis of hepatocellular carcinoma.

4 Epidemiological Studies

Researchers or scientific authorities adopt the view that nutrition can reduce the risk of cancer. 80% of cancers are associated with environmental factors, only 1% are caused by cancer syndromes and up to 5% are caused by single gene mutations. Therefore, it is predicted that 35–40% of cancers can be prevented by nutrition and physical activity (Wilson et al. 2002; Tandon et al. 2008). Although several epidemiological studies provide evidence that garlic consumption changes the course of the disease by affecting the molecular pathogenesis of cancer, long-term intervention studies are lacking. For example, stomach cancer mortality was 13 times lower in those who consumed 20 g of garlic per day than those who consumed only 1 g/day in Shandong province of China (Han 1993). According to the Chinese Academy of Medical Sciences, there was a negative relationship between the consumption of

garlic with the incidence of gastric cancer (Setiawan et al. 2005; Li et al. 2018a). Kodali and Eslick (2015) also reported a significant association with an elevated allium consumption and reduced gastric cancer risk in a meta-analysis study consisting of 8621 cancer cases and 14,889 controls. In a case-control and meta-analysis study of 230 cancer cases and 547 controls, a negative relationship was reported between increased garlic consumption and reduced risk of gastric cancer (Turati et al. 2015). However, in a study conducted in the Korean population, there was no significant relationship between garlic intake and decreased stomach cancer incidence (Kim et al. 2002). Dorant et al. (1996) and You et al. (2006) also adopted the same view. In another study in China, the intake of more than 10 g daily of allium vegetables in men reduced risk of prostate cancer compared to those who used less than 2.2 g daily (Hsing et al. 2002). Kirsh et al. (2007) reported that more than once intake per week was not associated with prostate cancer risk. In the case-control study conducted in China, it was observed that consumption of raw garlic more than twice [OR of 0.78 (95% CI: 0.62–1.01)] per week was negatively related to risk of liver cancer (Liu et al. 2019). These outcomes explain that daily dose should be determined according to cancer types in reducing the incidence.

Several studies have demonstrated that high garlic consumption is negatively related to the risk of prostate (Salem et al. 2011; Zhou et al. 2013), esophagus (Chen et al. 2004), larings, ovarian, renal and oral (Galeone et al. 2006), breast (Desai et al. 2019), multiple myeloma (Wang et al. 2012a), endometrium (Galeone et al. 2009), liver (Zhang et al. 2013), primary invasive epithelial ovarian and colon cancer (Steinmetz et al. 1994; Levi et al. 1999; Galeone et al. 2006). One of the most impressive studies have revealed that when garlic is consumed over 10 years, the incidence of hematological malignancy can lead to a 45% reduction (Nicastro et al. 2015). Nevertheless, there are studies claiming that there is no significant relationship between garlic consumption and cancer incidence. For instance, it was stated that the use of garlic is not related to the risk of colon (Tanaka et al. 2004; Giovannucci et al. 1994), lung (Dorant et al. 1995) and breast cancer (Galeone et al. 2006). In another study, after topical ajoene application in 21 patients with nodular or superficial basal cell carcinoma, it was reported that tumor size was reduced in 17 patients, Bcl-2 expression was significantly reduced, thus mitochondria-mediated apoptosis was stimulated (Tilli et al. 2003).

In some studies showing that garlic consumption has decreased cancer incidence, it was understood that the number of subjects was low, a low dose-control group was used instead of the placebo group, or the garlic intake was determined with qualitative questions without quantitatively measuring. On the other hand, the results may be directly related to the countries' diet (cooked or raw) and consumption amounts, and the reason for the differences between studies. Therefore, there is a need for advanced epidemiological studies based on larger populations and quantitative data.

5 Mechanisms of Action

5.1 Stimulation of Detoxification Enzymes

One of the mechanisms mediating the anticarcinogenic effect of garlic and some allyl sulfur compounds is the induction of detoxification enzymes. In mammalian systems, these enzymes are generally divided into two classes, phase I and phase II enzymes. Phase I metabolism largely occurs through cytochrome P450 (CYP450s) enzymes. Xenobiotics including drugs, toxins, carcinogens, mutagenes and toxic chemicals are metabolized by the CYP450s. In the phase II, the metabolized products are conjugated with molecules such as glucoronic acid, sulfate and glutathione, so that they can be excreted from the body through gall and urine.

Various allyl sulfur compounds have been reported to suppress or activate the expression of CYP450 genes (Srivastava et al. 1997). This suppression or activation can provide some benefits, such as preventing DNA damage, removing various carcinogens from the body. In a study by Davenport and Wargovich (2005), it was determined that DAS and DADS decreased rat liver protein level, while propyl-derived compounds and water-soluble SAC were not effective. However, DAS has been shown to increase liver CYP1A1 and CYP1A2 protein levels in time and dose depending manner. In addition, Wargovich (2006) demonstrated that DAS and DADS are the compounds that block CYP2E1 protein synthesis but the compounds of propyl origin cannot show the same effect. DADS increased the expression levels of liver and intestine CYP2B1 and CYP2B2 in rat. Although DAS had similar effect with DADS in the liver, only CYP2B1/2 protein levels were increased in the intestine.

Nitrosamines (NA) are potential carcinogens that affect the risk of cancer in humans and play a role in increasing this risk. Suppression of nitrosamine formation has been proposed as one of the possible anticancer action mechanisms of garlic, and allyl sulfur compounds. (Atanasova-Goranova et al. 1997; Dion et al. 1997). They also regulate phase I and II enzymes and DNA repair (Wattenberg 1990). Several studies have shown that DAS is a competitive inhibitor of N-nitrosodimethylamine (NDMA), a highly carcinogenic NA (Yang et al. 2001; Fasolino et al. 2015). Studies have shown that SAC is more effective than DAS and DADS in suppressing nitrosamine formation (Dion et al. 1997; Milner 2001). These effects of allyl sulfur compounds can be associated with inhibition of carcinogen activation by the P450s.

Activation of detoxification pathways through the induction of phase II enzymes (glutathione S-transferase (GST), UDP-glucuronosyl transferase (UGT), quinone reductase) is suggested as one of the main anti-tumor mechanisms of allyl sulfide compounds (Hu et al. 1997; Andorfer et al. 2004). Although DADS significantly increases GST and glutathione (GSH) levels in rats, SAC does not show the same effect. It has been suggested that an allysulfur-rich diet can alter chemotherapy treatment by increasing the expression of genes associated with multiple drug resistance (Demeule et al. 2004). GSH activity has also been shown to increase in the

DAS-treated mice stomach (Maurya and Singh 1991). There is a positive relationship between chemopreventive effects of the allyl sulfur compounds such as DAS, DADS and DATS, and increased NAD(P)H:quinone oxidoreductase (NQO) expression in benzo(a)pyrene (BP)-induced forestomach and lung cancer (Singh et al. 1998).

Garlic OSCs are H_2S donors, gaseous signaling molecules, and release H_2S through mainly GSH-dependent mechanism. Also, some OSCs such as DATS perform this secretion much faster than others (Liang et al. 2015). Under physiological conditions, while endogenous H_2S or relatively low exogenous H_2S takes part of maintaining homeostasis or limiting tissue damage, prolonged or high amount of H_2S exposure is thought to cause cancer cell death due to cellular toxicity (Han et al. 2019). Therefore, exogenous H_2S sources could be used as powerful therapeutic agents against a variety of diseases, including cancer.

As a result, some CYP450 enzymes and GSH mediate the anticancer effect of allyl sulfur compounds. However, compounds carrying allyl and oil-soluble groups are more effective in stimulation of detoxifying enzymes than those carrying propyl and water-soluble groups (Chen et al. 2004). Moreover, some components are not effective at the mRNA level, but at the protein level. This is an example of translational-level mechanisms of action, including epigenetic mechanisms.

5.2 Cell Protection from Oxidative Stress

Increased intracellular level of reactive oxygen species (ROS) causing oxidative stress is closely related to the pathogenesis of many diseases, including cancer (Liou and Storz 2010). Antioxidant system (involved in enzymes such as glutathione peroxidase (GPx), GST, catalase, superoxide dismutase (SOD)) neutralizes the oxidative damage. Garlic and its allyl sulfur compounds display free radical scarvening activity and protect the cell from lipid, protein and DNA damage (Sowjanya et al. 2009; Upadhyay 2017). Oral ingestion of garlic in animal models has been shown to reduce lipid peroxidation, increase circulating antioxidants, reduce glutathione and glutathione peroxidase (Balasenthil et al. 2000), and exhibit antimutation effect against gamma radiation (Chang et al. 2012). SAC has been found to have antioxidant properties in *in vitro* and *in vivo* models, improve $K_2Cr_2O_7$-induced toxicity (Medina-Campos et al. 2007) and reduce DNA damage (You et al. 2006). Similarly, SAMC plays a role in reducing ROS formation, preventing DNA damage, increasing SOD activity, and preventing NF-κB activity (Wang et al. 2016). DADS and DATS have also been shown to fight cellular stress by activating antioxidant enzymes (Awan et al. 2019). However, DAS, DADS and DATS stimulate the production of ROS (Antosiewicz et al. 2006; Das et al. 2007) by triggering cellular apoptosis and arresting the cell cycle (Yang et al. 2009).

5.3 *Induction of Cell Death and Cell Cycle Arrest*

Possible anticancer activity mechanisms of garlic's OSCs are summarized in Fig. 11.3. Cellular death and regulation of the cell cycle are among the most studied anticancer mechanisms of garlic and its OSCs. Although the only cell death mechanism is considered to be apoptosis (type I cell death), autophagy (type II cell death) and necrosis (type III cell death) are also included in this classification nowadays. In multicellular organisms, the main cellular death mechanism is apoptosis which is required for homeostasis in the development process from embryonic period to aging. However, apoptosis is triggered by intracellular (caspases) and extracellular pathways (death receptors) in immune response or cellular damage (Norbury and Hickson 2001). On the other hand, autophagy is a process for cellular homeostasis and cell survival that involved the remove of misfolded or aggregated proteins and damaged organelles as well as eliminated intracellular pathogens.

In some studies, involving the effects of garlic allyl sulfur compounds on cell death, SAMC has been shown to inhibit cell growth in gastric cancer through apoptotic proteins (Katsuki et al. 2006) and trigger MAPK-induced apoptosis by TGF-β

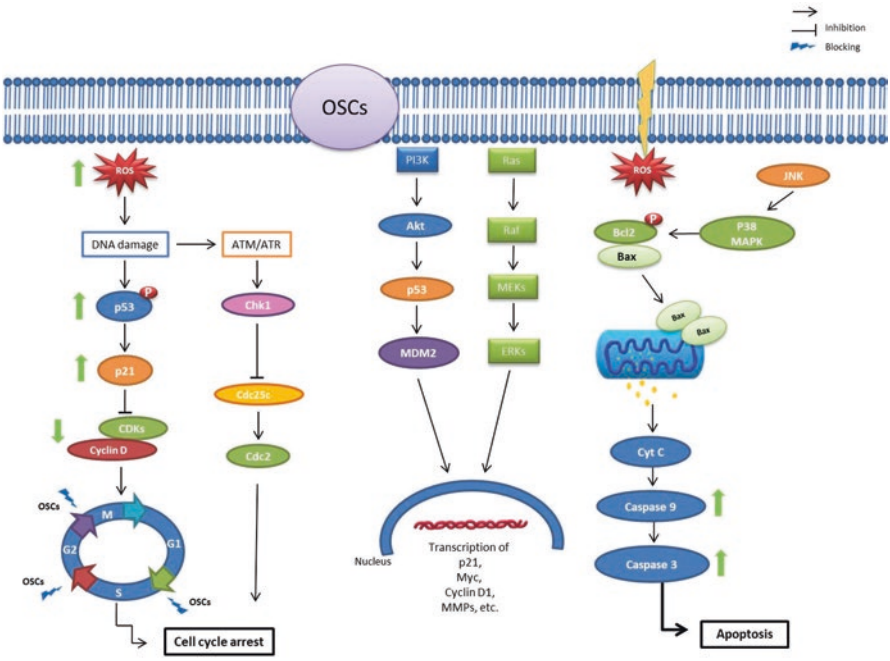

Fig. 11.3 Molecular mechanisms of action of OSCs-induced cell cycle arrest and apoptosis in cancer cells. OSCs induce ROS generation and DNA damage. It results the activation of phospho-53 and p21, and p21 inhibits the regulatory proteins, and then blocks the cell cycle at G1/S, S/G2 and G2/M. Also, OSCs activates ERK and PI3K for transcription of some survival genes such as p21, Cyclin D1. And they activate the JNK and p38 MAPK, and then upregulate of Bax and down-regulate of anti-apoptotic protein Bcl-2 gene. Decreasing of mitochondrial membrane potential trigger the release of Cyt C from mitochondria, and it results activation of caspase 9, caspase 3 and PARP that induce the caspase dependent apoptosis

activation in colon and hepatocellular carcinoma cells (Tong et al. 2014). Although SAMC is more effective than SAC, it is reported that it suppresses the proliferation and invasion of prostate, ovarian, nasopharyngeal and esophagal cancer cells and rearranges the cell cycle (Chu et al. 2006a). However, SAC has been shown to upregulate caspase-3 in ovarian cancer lines and inhibit cell proliferation by stimulating DNA methylation via DNA methyltransferases (DNMTs) (Xu et al. 2014, 2018). After SAC treatment, it was demonstrated that the antiapoptotic proteins Bcl-2 and Bcl-xL expression decreased and apoptotic proteins Bak and PUMA expression increased (Velmurugan et al. 2005; Ng et al. 2012). In HepG2 cells, it stimulates the apoptosis and cell cycle arrest through p53/p21 and JNK/c-Jun pathways (Knowles and Milner 2003). Based on these data, water-soluble allyl sulfur compounds promote cell death through both intracellular and extracellular apoptotic proteins.

Hong et al. (2000) found that DAS, DADS, and ajoen direct cancer cells to apoptosis by increasing the expression of apoptotic proteins (such as p53 and Bax) and decreasing the expression of antiapoptotic Bcl-2 through DNA fragmentation and intracellular free calcium. In addition, DADS inhibits cell proliferation by inducing cell cycle arrest in G2/M phase by decreasing cyclin B, Cdc2 and Cdc25C in ECA109 esophageal squamous cell line. Then caspase-mediated apoptosis accompanied by Bcl-2 and Bax proteins and inhibition of MAPK/ERK pathway takes place (Yin et al. 2014). Kelkel et al. (2012) suggested that DATS exhibits anticancer properties by inhibiting tubulin polymerization, in particular, this effect is related to the number of sulfur atoms.

Xiao et al. (2009) showed that DATS stimulates apoptosis by arresting cell cycle in G2/M phase through checkpoint kinase 1 (CDK1) by phosphorylation of its Tyr 15 residue in LNCaP and HCT-116 human cancer cells. On the other hand, DATS has been shown to be effective in preventing the angiogenic properties of human umbilical vein endothelial cells (Xiao et al. 2006b), stimulating human epidermal growth factor receptor2 (HER2) or p53-induced apoptosis, cell growth, migration and cell viability in MCF-7 and MDA-MB-231 breast cancer cell line (Antony and Singh 2011; Chandra-Kuntal et al. 2013).

The integrity of the cell cycle is essential to maintain healthy cell proliferation. It is mainly regulated by cyclin dependent kinases (CDKs) and inhibitors. Any problem in cell division can initiate the tumor process by causing uncontrolled cell divisions. Therefore, agents that inhibit tumor growth at phases of the cell division are among the therapeutic targets. It has been reported that SAMC, DAS, and DADS cause an increase in the percentage of cells blocked in the G2/M phase (Knowles and Milner 2001). In another study, DADS has been shown to block the cell cycle in the G2/M phase on osteosarcoma cells, thereby stimulating apoptosis and autophagy (Knowles and Milner 2001). This anticancer effect of DADS is due to the blocking of the phosphoinositide 3-kinase/mammalian target of rapamycin (PI3K/Akt/mTOR) signal pathway, one of the major pathways involved in the growth and proliferation of many cancer cell types (Yue et al. 2019). Similarly, Chu et al. (2013) suggested that allicin inhibits cell viability by decreasing the level of Bcl-2, cytoplasmic p53 and PI3K/Akt/mTOR signaling pathway and stimulates autophagy by

increasing tumor-suppressor AMP-activated protein kinase/tuberous sclerosis complex (AMPK/TSC2) expression and Beclin-1 signaling pathways.

5.4 Immune System Enhancement

Immune system is an incredibly complex host defence mechanism involved many biological structures and processes within an organism (Bourgeon et al. 2007). The host defense cells in the inflammatory system secrete many cytokines, chemokines and similar molecules to suppress malignant cells (Korniluk et al. 2017). When the defense system encounters a stimulus, it activates intracellular signaling pathways especially NF-kB, MAPK and JAK/STAT pathways, releasing inflammatory mediators (Chen et al. 2017). Expression of proinflammatory cytokines (such as IL1β, IL6, TNF-α) with the activation of these pathways supports tumor development. In addition, anti-inflammatory cytokines such as IL10 reverse this condition. Therapeutics that affect inflammatory pathways are being investigated in detail as they can potentially change the cancer process.

Evidence for both preventive and therapeutic effects of garlic on anticancer activity has been presented so far. Garlic and its OSCs have been shown to inhibit cancer progression by influencing inflammatory responses or by regulating cytokine production (Guan et al. 2018). For instance, garlic extract decreases the release of IL12, TNF-α, IL1α, IL6, IL8, IFNγ, IL2 cytokines, while increases the level of IL10 (Hodge et al. 2002).

It has been determined that SAC inhibits NF-κB activation in human T lymphocytes stimulated by TNF-α and H_2O_2 (Geng et al. 1997). In another study, it was reported that garlic extract inhibits NF-κB and the molecules in TLRs and LPS receptor signaling pathway cascades (Youn et al. 2008). DADS supresses NF-κB thought blocking the glycogen synthase kinase 3β (GSK-3β) activity, and inhibits tumor growth (Saud et al. 2016) in colitis-induced colorectal cancer, stimulates the release of IL1β, TNF-α and IL6, inhibits the release of IL10 in LPS-stimulated human whole blood (Keiss et al. 2003; Chang et al. 2005). As a result, garlic and its allyl sulfur compounds can regulate inflammation by modulating cytokines and leading to inhibition of NF-κB activity.

5.5 Epigenetic Mechanisms

Not only genetic mechanisms, but epigenetic mechanisms also get involved in the cancer process. Epigenetic mechanisms are all changes in gene expression without modifying the DNA base sequence. There are three defined mechanisms involved epigenetic: histone modification (acetylation, methylation, phosphorylation, ubiquitination and sumolation), DNA methylation at the transcriptional level and noncoding ribonucleic acid regulation at the post-transcriptional level. These

mechanisms affect the binding of transcription factors to DNA. For example; low level of methylation (hypomethylation) activates gene expression by DNA methyltransferase inhibitors (DNMTi), while its high level suppresses gene expression by preventing transcription factors from binding to the promoter. Hypomethylation is a common condition in the early stages of cancer. Therefore, agents targeting epigenetic mechanisms in developing cancer therapeutics remain the focus of attention. In addition, histone deacetylases (HDAC) are considered as potential drug targets because they affect cellular processes such as differentiation, apoptosis, angiogenesis, invasion, and metastasis. Studies show that garlic and various allyl sulfur compounds act as HDAC inhibitors and activate epigenetically silenced genes, leading to apoptosis and cell cycle arrest. Indeed, the allyl mercaptan and DADS are examples of potent HDAC inhibitors (Nian et al. 2009; Druesne-Pecollo and Latino-Martes 2011). DADS has been shown to inhibit cell proliferation related with increased $p21^{WAF1}$ expression, HDAC inhibition, and histone acetylation in colon cancer cell lines (Druesne et al. 2004a, b). DADS also stimulates cellular apoptosis as a result of increased histone acetylation in prostate cancer cells and inhibits the growth of H-RAS oncogene-transformed tumors (Singh et al. 1996). Apart from the fat-soluble allyl sulfur compounds, water soluble garlic extract has also been reported to inhibit tumor proliferation related with histone hyperacetylation in the T-cell lymphoma cell line (Bhuiyan et al. 2015).

6 Future Directions for Research on the Anticancer Effects of Garlic

Even though garlic and its organosulfur compounds have been used in food or pharmaceutical industry throughout history, detailed research has been undertaken for the past few decades involving mechanisms of action. It is very difficult to treat cancer after it has spread throughout the body which is called as metastasis. Various allyl sulfur type compounds were shown to have a decreasing effect on the frequency of cancer occurrence and progression. Therefore, they are potential agents in anticancer therapy, alone or in combination with antitumor drugs. Antioxidant, apoptotic, proliferative, cell cycle regulating, anti-inflammatory and detoxifying mechanism are among their described mechanisms of action. However, more studies are needed to understand the mechanism of action at both molecular and biochemical levels.

According to the literature, all isolated allyl sulfur compounds do not show the same anticancer effect when evaluated separately. In addition, garlic components, especially the lipophilic ones having allyl groups, exhibited higher anticarcinogenic activities via those mentioned mechanisms. However, there are many *in vitro* studies showing the anticancer properties of water soluble components, especially SAMC or allicin. Accordingly, when developing therapeutically effective compound/compounds from garlic or its preparations, this should be taken into

consideration and its clinical implications should be evaluated. Due to the fact that many of the recent studies do not contain quantitative results the data on garlic's effects on metabolism is limited. Although the studies carried out so far have provided sufficient data on a cellular basis, in clinical trials more attention should be drawn into the factors such as the applied dose, the route of administration and the type of the cancer as well as the diet style. It is important to know that individuals will have different respond to garlic intake, as the causes of cancers depend on variable factors including genetic and environmental. Detailed *in vitro* and *in vivo* studies on allyl sulfur compounds are still in need for further clarification between epigenetic mechanisms of action, especially in tumor inhibition, proliferation, invasion and metastasis.

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