Chapter 6
Cellular and Molecular Mechanisms of Garlic Compounds in Common GI Cancers

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Abstract The major gastrointestinal (GI) tract cancers are stomach, colorectal, pancreas, and liver cancers, which are foremost prevalent cancers worldwide, accounting for more deaths than any other cancers of human body. The GI tract cancers affect both men and women with preventable lifestyle risk factors including diet. Garlic is a globally used food ingredient with innumerable medicinal benefits due to the presence of sulfur-containing natural constituents such as alliin, methiin, DAS, DADS, DATS, SAC, and SAMC. They reduce GI cancer growth by inhibiting proliferation through disruption of microtubule-mediated cytoskeleton formation, inhibiting different cyclin/cyclin-dependent kinases in a phase-specific manner, and inducing apoptosis through mitochondrial dependent and independent pathways. The garlic compounds inhibit angiogenesis in GI cancers by downregulating VEGF, AKT/ERK, and NO signaling in tumor-induced endothelial cells. They also inhibit metastasis by inhibiting NF-κB and MMP2/9 signaling pathways. They exhibit antitumor by increasing the activity of NK cells, by secreting cytokine and chemokines, and by enhancing phagocytic activity of macrophages. Therefore, the consumption of garlic compounds may provide some kind of preventive mechanism against GI cancers through modulation of immune system.

Keywords Apoptosis · Gastrointestinal tract cancers · Garlic compounds · Proliferation and metastasis

Abbreviations

AGE	Aged garlic extract
AMC	Allyl mercaptan
COX2	Cyclooxygenase-2
CYP	Cytochrome P450

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DADS	Diallyl disulfide
DAS	Diallyl sulfide
DATS	Diallyl trisulfide
DNMT1	DNA methyltransferase 1
DVLS-2	Disheveled-2
EMT	Epithelial-mesenchymal transition
FGF-2	Fibroblast growth factor-2
FN	Fibronectin
GSAC	γ-Glutamyl-S-allyl-L-cysteines
HDAC	Histone deacetylase
HIF	Hypoxia-inducible factor
HMG-CoA	β-Hydroxy β-methylglutaryl-CoA
HUVAC	Human umbilical vein endothelial cells
IFN-gamma	Interferon-gamma
IL-2	Interleukin 2
JNK1	c-Jun N-terminal kinases
LEF-1	Lymphoid enhancer factor
MAP kinase	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
MDM2	Mouse double minute 2 homolog
MDR1	Multidrug resistance protein 1
MMP-2	Matrix metalloproteinase-2
MRP1	Multidrug resistance-associated protein 1
MSI	Microsatellite instability
NK cells	Natural killer cells
NO	Nitric oxide
Nrf-2	Nuclear factor erythroid 2-related factor 2
OSC	Organosulfur compound
PARP	Poly (ADP-ribose) polymerase
PCD	Programmed cell death
ROS	Reactive oxygen species
SAC	S-allyl cysteine
SAMC	S-allyl mercaptocysteine
SPRC	S-propargyl-L-cysteine
STAT-3	Signal transducer and activator of transcription 3
TAMs	Tumor-associated macrophages
TGF-alpha	Transforming growth factor-alpha
TIMP	Tissue inhibitor of metallopeptidase
TNF-alpha	Tumor necrosis factor-alpha
VEGF	Vascular endothelial growth factor
VEGFR-2	Vascular endothelial growth factor receptor-2
VHL	von Hippel-Lindau

1 Introduction

Gastrointestinal tract (GI) cancers are a group of malignancies of GI tract and accessory organs. The etiological causes of GI cancers are primarily preventable lifestyle habits including diet, exercise, alcohol and tobacco, and sanitation. Globally, GI tract cancers are one of the foremost prevalent cancers, diagnosed in more than four million new cases every year, and affect both men and women. The major GI cancers including stomach, colorectal, pancreas, and liver cancers account for more deaths than any other cancers of human body [1]. Worldwide, these cancers are foremost medical and economic burden to patients in both developed and developing countries. Genomic biomarkers have been recognized as valid genetic tools for diagnosis as well as treatment of GI tract cancers [2]. Microsatellite instability (MSI) is recognized as a most promising marker for prognosis and prediction of GI cancers [3, 4]. Also, genotyping of tumors [5] and RAS/BRAF [6], PI3K/Akt [7], Wnt/ß-catenin, and STAT-3 are recognized as important markers of GI tract cancers [8, 9]. Further, genome and epigenome-based biomarkers for GI tract cancers were discovered using high-throughput technology. *RAS/BRAF mutant* genes are predicted as prognostic markers in colon cancer. However, MSI has been demonstrated as a most promising marker for colon cancer. Yu and Cheung proposed MSI as a prognostic biomarker of adenocarcinoma of pancreas [10]. Even though extensive efforts are devoted to develop novel drugs and diagnostic markers, the prognosis of advanced GI cancers is very poor. Large body of experimental as well as epidemiological studies has provided ample evidence to support associations of prevention and reduction of cancer risk with intake of essential cooking ingredients. Garlic is one of the commonly used ingredients of dishes and an extensively used natural remedy in folk medicines. The immunomodulatory and antioxidant activities of garlic are related to anticancer activity against several cancers [11].

2 Health Benefits of Garlic

Garlic has health benefits mainly by sulfur-containing organic compounds as well as their derivatives. The medicinal claims of garlic are treatment of leprosy, diarrhea, constipation, and infections. Garlic can be used as expectorant, antispasmodic, antiseptic, and antihypertensive agent. Further, garlic can be used as bactericidal [12], antibiotic [13], and antifungal [14] agent. Additionally, garlic can reduce chronic bronchitis [15], infections of upper respiratory tract [16], as well as influenza [17, 18]. It can also diminish sugar levels in blood [19] and risk of heart diseases [20, 21]. The most compelling studies reported significant correlation between reduction of risks of GI tract cancers and intake of garlic [22–25].

3 Biologically Active Compounds of Garlic

Garlic is a globally used spice with innumerable medicinal benefits. The most important sulfur-containing natural constituents of fresh garlic are S-ally-L-cysteine sulfoxide (alliin), S-methylcysteine sulfoxide (methiin), γ-glutamyl-S-allyl-L-cysteines (GSAC), and S-allylcysteine. Allicin is a typical garlic compound with pungent smell formed from alliin by allinase during crushing or cutting of garlic [12, 13, 26]. It is highly unstable and rapidly converted to diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), as well as diallyl tetrasulfide. Allicin, ajoene, allyl propyl disulfide (APDS), DAS, DADS, DATS, S-allyl cysteine (SAC), and S-allyl mercaptocysteine (SAMC) are prominent biologically active compounds of garlic [27]. The prominent organosulfur compounds of garlic and their biological activities of garlic compounds are depicted in Table 6.1.

3.1 Inhibition of Tumor Growth

Organosulfur garlic compounds inhibit proliferation of different human cancer cells [28] including prostate [29], skin [30], colorectal [31], lung [32], neuroblastoma [33], and melanoma [34] cancers. SAMC inhibits growth of colorectal cancer [35] by disruption of microtubules, which are required for the formation of cytoskeleton, and mitotic spindle, which is required for cell division [36]. DADS suppresses H-ras oncogene-containing tumor growth in xenograft model through decreasing the activity of HMG-CoA reductase and by inhibiting binding of p21 to membrane without affecting farnesyl transferase activity [37].

3.2 Inhibition of Cell Cycle

Cell cycle involves simulation of growth, replication, and division, controlled by checkpoints through diverse signal transduction pathways [38, 39]. The checkpoints witness the completion of events in each phase of cell cycle during genomic instability and DNA damage [38, 39]. Most commonly used anticancer agents primarily target cell cycle and interfere with different phases depending on cells, mode of action, as well as target. Garlic-derived compounds can suppress colon cancer cell proliferation by arresting cell cycle [40] through decreasing Cdk1/cyclin B1 activity, disrupting Cdk1 and cyclin B1 complex, and decreasing Cdc25C expression [41]. DATS mediates cell cycle arrest in G2/M phase due to oligosulfide chain (OSC) length [41–44]. In PC-3 cells, DATS mediates cell cycle arrest by increasing phosphorylation of Cdk1 at Tyr 15, inhibiting Cdc25C activity of Cdk1/cyclin B1 complex, increasing phosphorylation at inhibitory site (Ser216), as well as downregulating Cdc25C protein level [45]. It also induces mitotic arrest by altering tubulin network and chromatin condensation as well as by increasing histone H3

Table 6.1 Summary of anticancer activity of allyl sulfides against GI tract cancers

Garlic compound	Mechanism of action of garlic compounds			
	Gastric cancer	Colon cancer	Liver cancer	Pancreatic cancer
DADS	• Inhibits migration and invasiveness • Inhibits MMP-2 and -9 activity • Represses claudin proteins • Induces apoptosis • Decreases Bcl-2 expression • Enhances Fas and Bax expression • Increases caspase-3 activity	• Inhibits proliferation • Enhances apoptosis • Targets ECM proteins • Reduces metastasis • Targets MMP-2, −7, and -9 • Modulates PI3K, Ras, MAP kinases, ERK1/2, JNK1/2, pathways • Enhances early apoptosis • Enhances genomic DNA degradation • Induces cell cycle arrest • Enhances ROS levels • Increases cyclin B1 activity	• Affects proliferation and viability • Induces apoptosis • Activates MAPK pathway • Enhances intracellular ROS • Induces dysregulation of mitochondrial membrane potential • Triggers DNA damage • Induces G2/M cell cycle arrest • Increases mitochondrial apoptotic pathway	• Invasion and migration ability • Protects against cerulein-induced acute pancreatitis
Allicin	• Induces apoptosis • Activates caspase-3 • Activates p38 MAP kinase signaling pathway • Induces mitochondrial dependent apoptosis • Enhances Fas/Fas ligand-dependent apoptosis	• Induces cytotoxicity • Promotes apoptosis • Increases Nrf2 expression	• Induces genotoxicity • Inhibits CYP enzymes • Induces phase II enzymes • Sensitizes HCC cells to 5-FU-induced apoptosis • Ameliorates tamoxifen-induced liver injury • Induces p53-mediated autophagy	• Effectively induces apoptosis • Induces caspase-3 expression • Causes DNA fragmentation • Inhibits cell cycle • Induces p21 (Waf1/Cip1) cyclin-dependent kinase inhibitor expression • Enhances ROS generation
SAMC	• Inhibits tumor growth • Induces apoptosis • Modulates	• In combination with rapamycin, induces apoptosis • Upregulates Bax/Bcl-2 ratio	• Inhibits metastasis • Targets Ki-67 and PCNA • Induces cell cycle arrest at S/G2	–

(continued)

Table 6.1 (continued)

Garlic compound	Mechanism of action of garlic compounds			
	Gastric cancer	Colon cancer	Liver cancer	Pancreatic cancer
	MAPK and PI3K/Akt signaling pathways • Induces depolymerization of microtubule • Activates JNK-1	• Inhibits autophagic activity • Promotes MAPK inhibitor-induced apoptosis	transition • Induces apoptosis • Downregulates Bcl-xL and Bcl-2 proteins • Activates caspase-3 and -9 • Downregulates Cdc25c, Cdc2, and cyclin B1	
DAS	• Protects from MNNG-induced damages • Inhibits cytochrome P450 2E1	• Exhibits chemopreventive activity by increasing G2/M arrest • Increases STAT1-mediated PCD • Upregulates NF-kB expression • Increases caspase-3 activity • Suppresses ERK-2 activity • Promotes expression of drug-resistant gene MDR1 • Promotes expression of MRP3 gene	• Prevents initiation of estrogen-induced cancer • Protects against N-nitrosodiethylamine-induced tumorigenesis • Modulates testosterone-induced oxidative stress • Displays antigenotoxic activity • Prevents hepatocarcinogenesis	–
DATS	• Enhances chemosensitivity by attenuating NF-kB activity	• Enhances MRP1 expression • Inhibits NF-κB pathway • Hamper COX-2 pathway	• Enhances caspase-3-dependent apoptosis • Reduces viability of J5 liver cancer cells • Enhances G2/M phase arrest	• Enhances caspase-3-dependent apoptosis • Reduces viability of J5 liver cancer cells • Inhibits cell proliferation • Induces caspase-3 activity

phosphorylation at serine 10 in PC cells [42]. DATS also arrests cell division at prometaphase of PC-3 cells by activating Chk1 and by accumulating APC/C and cyclin A B1 along with hyperphosphorylation of securin [43]. DADS and SAMC also induce mitotic attest in PC-3 cells [43]. DATS mediates cell cycle arrest through generation of ROS in a JNK-dependent pathway [Ref]. In colorectal cancer cells, DATS induces mitotic arrest in mitotic cells by disrupting the network of microtubules as well as inhibiting the formation of spindle via oxidation-dependent tubulin β (cysteine-12 and -354) modifications [46]. The summary of the mechanism of DATS-mediated G2M arrest of cell cycle is depicted in Fig. 6.1.

Ajoene causes cells cycle arrest at G2/M phase by disrupting microtubule network and inhibiting tubulin polymerization [47]. DADS also mediates cell cycle arrest at S phase [48]. The synthetic derivative of DATS, allitridi, arrests cell cycle in G1 phase by decreasing cyclin D1 level and increasing p27 protein level in gastric cancer cells [49]. The arrest of cell cycle progression by garlic compounds can be mediated by histone modifications. The garlic compound-dependent histone acetylation affects cancer cell proliferation by regulating gene expression. For instance, DADS enhances H4 and H3 histone acetylation, but inhibits deacetylases [50]. Allicin, SAMC, and SAC inhibit colon cancer growth by increasing acetylation of histones [51]. The DADS induces cell cycle of colorectal cancer cells in G2/M phase by inhibiting hyperacetylation of histones H3 and H4, and histone deacetylase, and upregulating p21 levels [52]. DADS also affects cell cycle by decreasing tumor cells at the G1 and S phases with concomitant increasing of G2/M phase [40]. DADS is known to reduce proliferation of cells by inducting cell cycle arrest through inhibition of p34cdc2 kinase [41]. It also inhibits growth of implanted H-ras-dependent tumors by preventing the interaction of p21H-ras with cell membrane in nude mice [37]. The summary of garlic compound-mediated cell cycle arrest is presented in Fig. 6.1.

3.3 Apoptosis

Apoptosis/programmed cell death (PCD) with conserved and tight regulation is essential for normal development of embryo as well as maintenance of tissue homeostasis. Deregulation of apoptosis is the basis for various pathological states of cancer. Hence, apoptosis is an effective target for cancer treatment as well as prevention [53, 54]. The garlic compounds majorly mediate intrinsic or mitochondrial dependent apoptosis by promoting dissipation of mitochondrial membrane potential ($\Delta\Psi m$) along with release of apoptotic mediators into cytosol [55, 56]. The ultimate fate of the mitochondrial dependent apoptosis depends on the levels of anti-apoptotic (Bcl-2 and Bcl-xL) as well as pro-apoptotic (Bax and Bak) proteins of Bcl-2 family [57].

Garlic-derived compounds trigger PCD by modulating Bcl-2 protein levels. For instance, DAS and DADS increase Bax/Bcl-2 ratio in lung cancer cells [58, 59]. DADS treatment also upregulates Bax level with concomitant

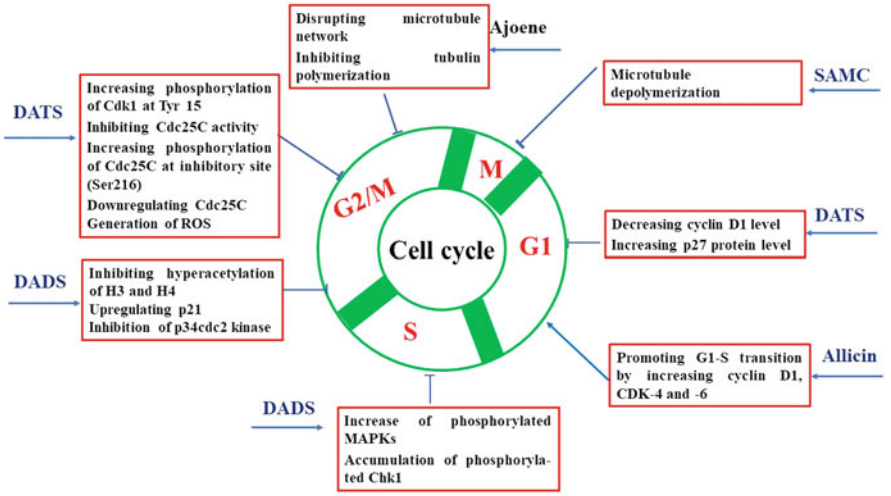

Fig. 6.1 Mechanism of garlic compounds on cell cycle. DATS induces cell arrest at G2M phase by increasing phosphorylation of Cdk1 at Tyr 15, inhibiting Cdc25C activity, increasing phosphorylation of Cdc25C at inhibitory site (Ser216), downregulating Cdc25C and generation of ROS, and inducing cell cycle arrest at G1 phase by decreasing cyclin D1 and increasing p27 protein levels. DADS causes cell cycle arrest at G2M phase by inhibiting hyperacetylation of H3 and H4, upregulating p21, and inhibiting p34cdc2 kinase and S phase by increasing phosphorylated MAPKs and accumulating phosphorylated Chk1. SAMC inhibits cell cycle at M phase by inducing depolymerization of microtubules. Allicin promotes G1-S transition by increasing cyclin D1, CDK-4, and -6. Ajoene induces G2M cell arrest by disrupting microtubule network and inhibiting tubulin polymerization

downregulation of Bcl-xL [60]. DAS and DADS increase apoptosis by enhancing p53 and Bax expression and decreasing Bcl-2 expression [59]. DADS and DATS induce apoptosis by changing the morphology as well as by causing fragmentation of DNA [31, 32]. DADS induces DNA fragmentation by increasing intracellular Ca^{2+} and activating Ca^{2+}-dependent endonucleases.

DATS is more potent in inducing apoptosis compared to other oil-soluble garlic compounds [61]. It induces apoptosis by decreasing expression and JNK-dependent hyperphosphorylation of Bcl-2, which decreases Bcl-2:Bax association and promotes intrinsic apoptotic pathway [61]. DATS also enhances PCD by increasing the expression of Bax as well as Bak [62]. DATS stimulates apoptosis mainly by controlling Akt-mediated Bad pathway [63]. Akt enhances sequestration of Bad in cytosol by phosphorylation and consequently reduces interaction of Bad with Bcl-2 protein. In fact, DATS reduces Akt-dependent phosphorylated Bad (Ser155 and Ser136) levels, thereby diminishing Bad and 14-3-3β interaction [63]. It is experimentally demonstrated that ROS is an intermediary of garlic-induced apoptotic cell death mechanisms. DADS induces cell death by generating ROS [64] via activation of JNK [65]. OSC induces apoptosis by increasing intracellular calcium. They induce release of intracellular Ca^{2+} along with hydrogen peroxide level and activate

caspase-3 [31, 32, 66, 67]. DAS and DADS can activate calpain by increasing calcium levels [58]. The Z ajoene promotes apoptosis by caspase-dependent cleavage of Bcl-2 via generation of ROS [68]. SAMC can also induce apoptosis by triggering activation of caspase cascade [36]. The mechanism of garlic compound-induced apoptosis is summarized in Fig. 6.2.

4 Antimetastatic Activity

Angiogenesis is indispensable for tumor growth beyond 1 mm in diameter [69]. Recent reports demonstrated that garlic-derived compounds inhibit tumor-induced angiogenesis and metastasis in cellular and animal models (Fig. 6.3). AGE inhibits proliferation and invasiveness of the endothelial cells by increasing cell adhesion to collagen and fibronectin [70]. AGE reduces endothelial cell-mediated formation of capillary tubes [70]. Even DATS is more efficacious in reducing the viability of HUVEC by increasing active caspase-3 and cleaving PARP as well as apoptosis [71]. DATS mediates reduction of capillary tube formation as well as migration of HUVEC by suppressing the secretion of VEGF, downregulating the expression of VEGFR-2, and inactivating Akt and activating ERK ½ [71]. Alliin also reduces VEGF- and FGF-2-mediated angiogenesis [72]. DADS and DAS reduce MMP-2 and -9 expression [73]. Alliin inhibits FGF2- and VEGF-mediated angiogenesis by upregulating p53 expression and by enhancing the release of NO [72]. Ajoene inhibits metastasis by disrupting the vimentin network [74]. DAS is another OCS of garlic that increases circulatory antiangiogenic factors and IL-2 and TIMP in C57BL/6 mice implanted with B16F-10 melanoma cells [75]. It can also inhibit differentiation [73] and angiogenic features of HUVAC cells by inactivating Akt and downregulating VEGF and VEGF-R2 [71].

Taylor et al. [92] reported that ajoene significantly inhibited lung metastasis of cancer cells. Likewise, SAMC reduced the lung metastasis without effect on local metastasis [93]. DATS inhibited hypoxia-dependent hematogenous metastasis by reducing HIF-1α mRNA expression [76]. DADS suppresses cancer metastasis by SRC/Ras/ERK signaling-dependent upregulation of miR-34a [77]. It can also inhibit invasiveness and cancer metastasis by repressing tight-junction protein claudin and by inactivating invasive proteins MMP-2 and -9 [78]. DADS reduces gastric cancer cell motility and invasion by upregulating the expression of TIMP-1 and -2 [79]. DADS reduces FN-induced metastasis by reducing the activity of gelatinases. It suppresses FN-mediated EMT by enhancing the expression of E-cadherin and cytokeratin-18 and by reducing the expression of N-cadherin and vimentin as well as snail, slug, and twist. It inhibits DVLS-2 and LEF-1 by preventing β-catenin translocation into nucleus and by phosphorylation-dependent inhibition of glycogen synthase kinase-3β [80]. DADS suppresses metastasis by modulating MMP/TIMP ratio through blocking NF-κB and PI3K/AKT pathways [81]. DATS diminishes cancer progression and experimental metastasis by targeting metastasis-related

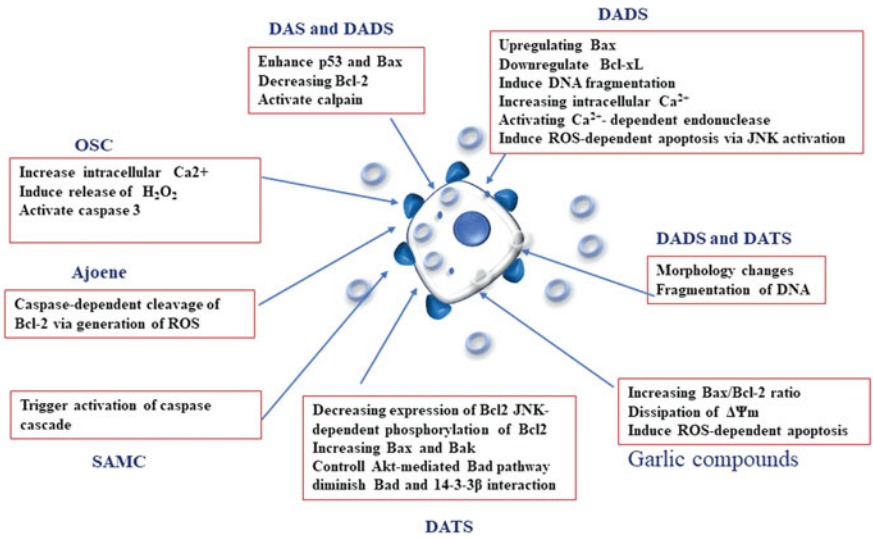

Fig. 6.2 Mechanisms of garlic compound-induced apoptosis. DAS and DADS enhance p53 and Bax, decrease Bcl-2 expression, and activate calpain. DADS upregulates Bax, downregulates Bcl-xL, induces DNA fragmentation, increases intracellular Ca^{2+}, activates Ca^{2+}-dependent endonuclease, and induces ROS-dependent apoptosis via JNK activation; DADS and DATS alter morphology and induce DNA fragmentation. DATS decreases the expression of Bcl2- and JNK-dependent phosphorylation of Bcl2, increases Bax and Bak, controls Akt-mediated Bad pathway, and diminishes Bad and 14-3-3β interaction. SAMC can trigger activation of caspase cascade. Ajoene enhances caspase-dependent cleavage of Bcl-2 via generation of ROS. Oligosulfur compounds (OSC) increase intracellular Ca^{2+}, induce release of H_2O_2, and activate caspase-3

genes, and NF-κB and MMP2/9 genes mediated by thioredoxin system [82]. DATS suppresses colon cancer stem cells by targeting colon spheres and stem cell markers via Wnt/β-catenin pathway [83].

5 Epigenetic Regulation

DADS inhibits cell cycle, induces apoptosis and autophagy, inhibits angiogenesis, and enhances ROS generation in cancer cells by modulating histone deacetylase (HDAC) [84]. It can reduce the metastasis of breast cancer cells by post-transcriptionally attenuating HIF-1α via von Hippel-Lindau (VHL)-dependent degradation [76]. Garlic can regulate gene expression by inhibiting histone deacetylase-mediated histone acetylation [85]. SAC inhibits proliferation of ovarian cancer cells by DNMT1-dependent methylation of DNA [86]. DATS increases the sensitivity of gastric cancer cells to docetaxel by diminishing NF-κB activity through epigenetic upregulation of metallothionein 2A [87]. These studies demonstrated that garlic compounds regulate gene expression through epigenetic mechanism.

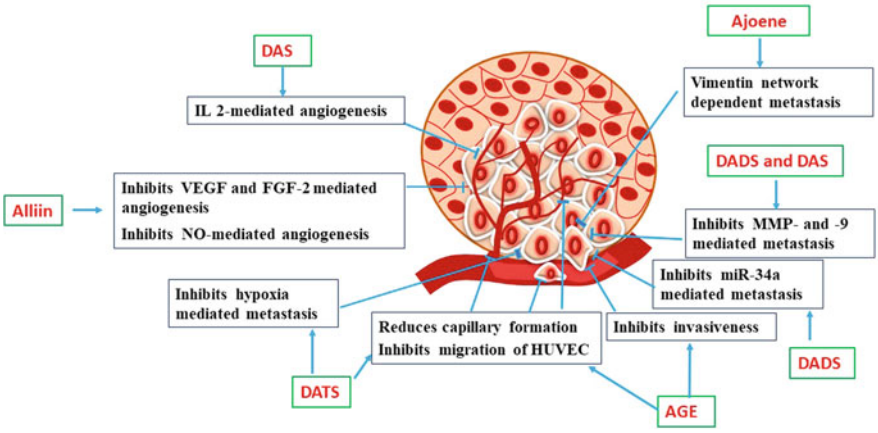

Fig. 6.3 Effect of garlic compounds on metastasis. DAS inhibits IL-2-mediated angiogenesis. DATS inhibits hypoxia-mediated metastasis, and reduces capillary formation and migration of HUVEC. AGE inhibits invasiveness of cancer cells and capillary formation of endothelial cells. DADS inhibits miR-34a-mediated metastasis. DADS and DAS inhibit MMP- and -9-mediated metastasis. Ajoene inhibits vimentin network-dependent metastasis. Alliin inhibits VEGF- and FGF-2-mediated and NO-mediated angiogenesis

6 Antitumor Immunity

AGE (500 mg/day) increases the activity of natural killer (NK) cells in advanced hepatic cancer patients [88]. ABGE-treated gastric cancer cells exhibit antitumor and immunomodulatory activity [89] by secreting IL-2, TNF-alpha, and IFN-gamma, by increasing the activity of NK cells and by enhancing the phagocytic activity of macrophages [90]. Garlic compounds prevent cancer by modulating immune response [91]. DADS inhibits cancer metastasis through modulation of tumor-associated macrophages (TAMs) via suppression of TNFα-mediated release of MCP-1 [92]. These studies support the anticancer activity of garlic compounds through immunomodulation.

The organic sulfuric compounds present in the garlic inhibit major GI tract cancers through different mechanisms. They exhibit stronger antitumor activity against GI malignancies by inhibiting the expression of oncogenes controlling tumor cell proliferation, cell cycle regulation, apoptosis, metastasis, and antitumor immunity (Table 6.1).

7 Antitumor Mechanisms in Gastric Cancer

DADS induces inhibition of migration and invasiveness by enhancing tightness of the tight junctions, and transepithelial electrical resistance [79]. It inhibits MMP-2 and -9 activities along with repression of claudin proteins (claudin-2, -3, and -4). DADS decreases gastric cancer cell growth by inducing apoptosis via decreased Bcl-2 expression-enhanced Fas, and Bax expression, as well as increased activity of casp-3 [93]. Allicin induces apoptosis by activating caspase-3 via p38 MAP kinase signaling pathway [94]. SAMC inhibits human gastric cancer growth in xenografts by inducing apoptosis through modulating MAPK and PI3K/Akt signaling pathways [95]. SAMC can induce apoptosis by depolymerizing microtubule and activating JNK-1 [36]. Allicin induces both mitochondrial dependent intrinsic and Fas/Fas ligand-dependent extrinsic apoptosis pathways in gastric cancers [96]. Garlic oil inhibits proliferation of gastric cancer cells by targeting the expression of cyclin E and autocrine and paracrine loops of TGF-α [97]. Further, combination of garlic oil and resveratrol prompts apoptosis synergistically in gastric cancer cells by increasing Fas and Bax and decreasing Bcl-2 expression [98]. SAMC inhibits gastric cancer cell growth by causing dose-dependent reduction of proliferation and induction of DNA fragmentation and caspase-3 activity via Bax and p53. It inhibits implanted gastric tumors in nude mice by regulating Bcl-2 and Bax expression [99].

8 Antitumor Mechanisms in Colorectal Cancer

Organosulfur garlic compounds are also reported to target metastasis. DADS reduces colorectal cancer growth by inhibiting proliferation and enhancing apoptosis via targeting extracellular matrix proteins [100]. For instance DAS, DADS, and DATS reduce metastasis by targeting MMP-2, -7, and -9 via modulating PI3K, Ras, MAP kinases, ERK1/2, and JNK1/2 pathways [101]. DADS reduces development of colorectal tumors along with dietary factors such as short-chain fatty acids/polysaccharides by reducing cell proliferation, enhancing early apoptosis, activating caspase-3 and -9, and enhancing genomic DNA degradation as well as cell cycle arrest [102].

Allicin induces cytotoxicity and apoptosis via increased expression of Nrf2 transcription factor. DADS inhibits proliferation of colon cancer cells by enhancing ROS-dependent G2/M arrest of cell cycle via increased activity of cyclin B1 and apoptosis by activating p53 [103]. Allyl sulfides modulate the activity of histone deacetylases. Allyl mercaptan (AM) is most potent in inhibiting the activity of histone deacetylase compared to its precursors, DADS and SAMC [104]. AM induces G1-phase arrest by increasing the p21 expression in colorectal cancer cells [105]. DAS exhibits chemopreventive activity by increasing G2/M arrest and STAT1-mediated PCD as well as upregulating NF-kB and caspase-3 and

suppressing ERK-2 activity [106]. DADS treatment significantly raises the intracellular Ca^{2+} by enhancing Ca^{2+} influx.

DAS, DADS, and DATS promote the expression of drug-resistant gene multidrug resistant 1 (MDR1) while DAS and DADS promote the expression of MRP3 gene, whereas DATS alone enhances the expression of MRP1 in colorectal cancer cells. However, DADS and DATS induce the expression of MDR1 and MRP1 genes, DADS promotes MRP3 gene while DADS and DATS increase MRP4 and MRP6 genes in in vivo xenograft model [107]. DATS inhibits NF-κB and COX-2 pathways [107]. These observations suggest the antimetastatic proliferation of colon cancer cells by targeting potentials of organosulfur garlic compounds.

9 Antitumor Mechanisms in Liver Cancer

SAC inhibits metastasis of liver cancer cells by targeting Ki-67 and PCNA and inducing cell cycle arrest at S/G2 transition [108]. It also induces apoptosis by downregulating Bcl-xL and Bcl-2 proteins and activating caspase-3 and -9. Moreover, SAC enhances S-phase cell arrest by downregulating Cdc25c, Cdc2, and cyclin B1. DATS showed significantly high anticancer activity against HepG2 cells in caspase-3-dependent apoptosis compared to DAS and DADS [109]. Similarly, DATS reduces viability of J5 liver cancer cells by enhancing the arrest of cells at G2/M phase. DATS-treated group displays significant number of G2/M arrest cells compared to DADS- and DAS-treated groups with increased Cdk7 and cyclin B1 protein levels due to difference in the allyl groups [110]. Water-soluble garlic extracts induced significantly marked effects on HepG2 cells compared to oil-soluble extracts [111]. They induce p53/p21-mediated G2/M arrest of cells and JNK-dependent apoptosis [112]. DADS affects proliferation and viability of hepatic cells by inducing apoptosis through activation of MAPK pathway [112]. Allicin, DAS, DADS, SAC, and AM induce genotoxicity by inhibiting CYP enzymes and inducting phase II enzymes [113]. SAC along with cisplatin inhibits tumor progression and metastasis of liver cancer cells in orthotopic xenograft [113]. Garlic oil reduces N-nitrosodiethylamine (NDEA)-induced liver cancer by decreasing Bcl-2, Bcl-xl, and β-arrestin-2 as well as increasing Bax and caspase-3 [114]. DMBA-induced liver carcinogenesis was prevented by DAS [115, 116].

10 Antitumor Mechanisms in Pancreatic Cancer

DATS reduces the viability of pancreatic carcinoma cells by enhancing G2/M phase and apoptotic cells via increasing Fas, p21, p53, and cyclin B1 expression and decreasing Akt, cyclin D1, MDM2, and Bcl-2 expression [117]. It also increases cleaved caspase 3 and PARP as well as Bim-s and Bim-L isoforms in apoptotic pancreatic cells [117]. S-propargyl-L-cysteine (SPRC) reduces pancreatic cancer

growth by inhibiting proliferation and promoting G2/M cell arrest and JNK-dependent apoptosis by enhancing its phosphorylation and by reducing its ubiquitin-dependent degradation [118]. Garlicin at higher concentration inhibits pancreatic tumor growth, while at lower concentration reduces cancer cell invasion and migration via targeting PI3K/AKT signaling pathway [119]. Allicin enhances apoptosis in pancreatic cancer cells by increasing caspase-3 activity, DNA fragmentation, and cell cycle arrest also inducing the expression of p21 (Waf1/Cip1), generation of ROS, and depletion of GSH [120]. Garlic oil shows remarkable inhibition of pancreatic cancer cell proliferation by accumulating cells at G2M phase and presenting significant level of apoptosis [121].

11 Conclusion

In recent past, there has been an increase in research on the impact of garlic and its derivatives in the treatment of various cancers especially GI and associated cancers. The sulfur-containing garlic compounds target multiple cellular mechanisms including proliferation, cell cycle, apoptosis, metastasis, and angiogenesis, which infer their anticancer activities. Garlic compounds also regulate gene expression through modulation of genes controlling epigenetic mechanisms. Further, limited studies demonstrated the antitumor immunity especially aged garlic extract. Additional information on cellular and molecular mechanisms of garlic compounds is required to understand their cancer-preventive mechanism in clinical studies.

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