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REVIEW

Potential antitumor activity of garlic against colorectal cancer: focus on the molecular mechanisms of action

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Abstract

Purpose The aim of this review is to highlight the potential of garlic phytoconstituents as antitumor agents in colorectal cancer management based on their molecular mechanisms of action, while asking if their consumption, as part of the human diet, might contribute to the prevention of colorectal cancer.

Methods To gather information on appropriate in vitro, in vivo and human observational studies on this topic, the keywords "*Allium sativum*", "garlic", "colorectal cancer", "antitumor effect", "in vitro", "in vivo", "garlic consumption" and "colorectal cancer risk" were searched in different combinations in the international databases ScienceDirect, PubMed and Google Scholar. After duplicate and reviews removal, 61 research articles and meta-analyses published between 2000 and 2022 in peer-reviewed journals were found and included in this review.

Results Garlic (*Allium sativum*) proves to be a rich source of compounds with antitumor potential. Garlic-derived extracts and several of its individual constituents, especially organosulfur compounds such as allicin, diallyl sulfide, diallyl disulfide, diallyl trisulfide, diallyl tetrasulfide, allylmethylsulfide, S-allylmercaptocysteine, Z-ajoene, thiacremonone and Se-methyl-L-selenocysteine were found to possess cytotoxic, cytostatic, antiangiogenic and antimetastatic activities in different in vitro and in vivo models of colorectal cancer. The molecular mechanisms for their antitumor effects are associated with the modulation of several well-known signaling pathways involved in cell cycle progression, especially G1-S and G2-M transitions, as well as both the intrinsic and extrinsic apoptotic pathways. However, even though in various animal models some of these compounds have chemopreventive effects, based on different human observational studies, a diet rich in garlic is not consistently associated with a lower risk of developing colorectal cancer.

Conclusion Independent of the impact of garlic consumption on colorectal cancer initiation and promotion in humans, its constituents might be good candidates for future conventional and/or complementary therapies, based on their diverse mechanisms of action.

Keywords Garlic · Colorectal cancer · Cytotoxic · Cytostatic · Antimetastatic

Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer morbidity and mortality worldwide, accounting for more than 900.000 deaths in 2020 [1]. Despite the significant progress made in CRC treatment in the past decades, its prevalence is still expected to rise over the next 10 years by 60%, with over 2.2 million new cases and 1.1 million deaths, making colorectal cancer a severe health concern [2].

The onset and progression of CRC involves various genetic and epigenetic changes affecting cell proliferation and differentiation, cell survival and apoptosis, tumor angiogenesis and metastasis, which in turn give tumors a selective advantage for survival and may cause current chemotherapy approaches to be ineffective [2, 3]. Classically, cancer therapies have been focusing on cytotoxic agents that kill actively dividing cancer cells. Unfortunately, this is not always attainable in effective doses as most agents often end up killing normal, healthy cells as well, determining several

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side effects [4]. Besides the severe side effects, multidrug resistance is another important problem in CRC treatments [5]. In this context, over the past 20 years new cancer therapies based on plant compounds were developed and proved to be effective and safe, without causing too much damage to normal cells. Researchers used both in vitro and in vivo model systems for new, non-toxic drug discovery [6], demonstrating the chemopreventive, cytostatic, cytotoxic and antimetastatic potential of several plant-derived compounds, mainly plants secondary metabolites [7].

Garlic (*Allium sativum*), a bulbous plant of Amaryllidaceae family, presents a mixture of complex metabolites including phenolics, flavonoids, carotenoids and alkaloids, as well as organosulfur compounds (OSCs) [8]. Among them, the bioactive components that most often appear to protect against colorectal cancer are OSCs, predominantly allyl derivatives, such as aliin, allicin, ajoene, diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), S-allylcysteine (SAC) and S-allylmercaptocystein (SAMC) [9–12]. Taking into consideration that garlic bulbs are part of the human diet and the high content of bioactive compounds found in them, several observational studies were carried out to assess the potential of garlic consumption in CRC prevention.

In 1991, Hu et al. [13] were among the first to show that consumption of garlic was associated with reduced risk of developing rectal cancer. Several other studies also showed that high consumption of garlic has a protective effect against colorectal cancer [14, 15]. In 2000, a meta-analysis based on these studies and four others provided evidence that garlic consumption decreases the CRC risk by approximately 30% [16–19]. However, other studies have yielded contrasting results, showing no association between garlic intake and the risk of colorectal cancer [20–23]. Since 2020, in more recent attempts to elucidate the impact of garlic consumption on CRC risk, three different meta-analyses have been performed, pooling together 11 [24], 9 [25] or 7 [26] observational studies. Interestingly, a consensus between these recent meta-analyses could not be reached, with two of them suggesting that overall, the consumption of garlic is associated with a reduced CRC risk [24, 25], whereas the third found no significant association between *Allium* vegetable consumption, including garlic, and the risk of colon cancer [26]. The main reason for such opposing results is represented by the inclusion criteria used in each of the three meta-analyses, a very different set of prospective/retrospective studies being analyzed. Therefore, all these results must be interpreted with caution and further large-scale observational studies/interventional trials are needed to confirm the effect [24].

Even though it is not yet clear if garlic consumption might decrease the risk of CRC, there is still great potential for garlic, as recent studies shed light on a novel mechanism for colorectal cancer prevention using garlic-derived compounds. Garlic, being a traditional part of the Mediterranean diet, presents chemopreventive properties by affecting the gut microbiome as well [27]. Two novel immune-enhancing garlic-derived compounds improve the immune system by upregulating the abundance of several microbiome populations of protective nature, fortifying the mucosal barrier, and exerting selective cytotoxicity against tumor cells. WSGP (water-soluble garlic polysaccharide) and PTSO (propyl-propane thiosulfonate) inhibit the expression of several pro-inflammatory mediators (TNF-α, IL-1β, IL-8, IL-17 and iNOS), and restore gut microbial alteration induced by dextran sulfate sodium (DSS) exposure by increasing *Bacteroidetes* and *Actinobacteria* and reducing *Firmicutes/Bacteroidetes* ratio [28, 29].

Furthermore, the available in vitro and in vivo data regarding the potential of *A. sativum* in CRC management are rather convincing, demonstrating substantial antitumor activities of different garlic-derived compounds and extracts [30–32]. Therefore, in this paper, we review the latest updates on *Allium sativum* antitumor activity, both in vitro and in vivo and the phytochemical constituents responsible for such biological activities, with a special focus on the cellular and molecular mechanisms of action. Furthermore, we try to explain the apparent contradiction between the in vitro/ in vivo data and the possible lack of association between garlic consumption and the risk of CRC development.

In vitro studies

In studying the mechanism of garlic's phytochemicals action and designing effective treatments, scientists have been using well-characterized colon cancer cell lines as a biological model. To assist in in vitro studies, ten CRC cell lines such as Caco-2, DLD-1, HCT-15, HCT116, HT-29, COLO 205, SW480 and SW620, were used over the years. Many in vitro studies have shown the antiproliferative (cytostatic), apoptosis/necrosis-inducing (cytotoxic), antimigration, antiinvasion and immune-modulating activities of garlic and its constituents against colorectal cancer. Table 1 presents the in vitro activity and the proposed mechanism of action of garlic extracts and its phytoconstituents used alone or in combination with nanoparticles or chemotherapeutic drugs.

Cytostatic effects

Several studies have demonstrated that many bioactive compounds found in garlic have cytostatic effects. The definition of a cytostatic drug states that these compounds are able to specifically inhibit cell cycle progression in different human cancer cells without actually killing the

Table 1 Main cellular processes affected by garlic-derived compounds in different colorectal cancer cell lines in vitro and their molecular mechanisms of action

Compound	Reported effect—cellular process affected	Molecular mechanisms	Tested cell lines	References
DADS	Cytostatic effects—cell cycle arrest in G2/M	Cyclin B1, PCNA, p53 ↓ p21 ↑	SW480	[35]
		CDK1/cyclin B ↓ CHK1, WEE1, CDC25C-ser-216 ↑ CDC25C ↓	COLO 205	[34]
		p21 ↑ +49 other cell cycle control genes	HT-29	[37]
		CDK1/cyclin B complex formation ↓ CDK1 kinase activity ↓ CDC25C phosphatase activity ↓	HCT-15	[32]
		p21 ↑ H3 and H4 acetylation ↑ HDAC deacetylase activity ↓	HT-29 Caco-2	[36]
		Cyclin B1, ROS ↑	HCT116	[50]
	Cytotoxic effect—apoptosis	Intracellular ROS ↑ p53 ↑ caspase 3 activity, PARP cleavage ↑	HCT116	[50]
		p53, NAG-1 ↑	HCT116	[52]
		Intracellular Ca^{2+}, H_2O_2 ↑ caspase 3 activity ↑	HCT-15	[74]
		Intracellular Ca^{2+} ↑	SW480	[49]
		NFkB, GSK3β, COX-2 ↓	SW480	[51]
		Intracellular Ca^{2+}, ROS ↑ p53, BAX, BAK, FAS, ASK1, JNK ↑ BCL-2, BCL-XL ↓ caspase 8/9/3 activity ↑	COLO 205 (IC_{50} = 22.47 μM)	[34]
		TRAIL-induced caspase activity ↑ BCL-2 ↓	HCT116 DLD-1 HT-29 SW620	[54]
	Inhibition of migration and invasion	p38, PI3K, ERK, JNK, NFkB ↓ MMP − 2, − 7, − 9	COLO 205	[68]
		LIMK 1, RAC1, ROCK1, PAK1 ↓	SW480	[70]
DADS + NaB	Cytotoxic activity—apoptosis	Caspase 9, 3 activity ↑	HT-29	[53]
DATS	Cytostatic—G1/G0 arrest and finally apoptosis	GSK3β ↑ β-catenin, cyclin D1, cMYC ↓ CD133, CD44, ALDH1, OCT-4, NANOG ↓	SW480 CSCs DLD-1 CSCs	[38]
	Cytostatic—G2/M arrest and finally apoptosis	Microtubule disarray	HCT-15 DLD-1	[39]
	Cytotoxic—apoptosis	ROS, BAX ↑ BCL-2 ↓ caspase 9/3 activity ↑	Human primary colon cancer cell	[56]
		BAX BCL-2 ↓ caspase 9/3 activity ↑	SW480 CSCs DLD-1 CSCs	[38]
	Inhibition of migration and invasion	FAK, C-JUN N-terminal kinase, p38 ↓ MMP-*2, -7, -9* ↓	HT-29	[69]
		p38, PI3K, ERK, JNK, NFkB ↓ MMP− 2, − 7, − 9 ↓	COLO 205	[68]
	Inhibition of angiogenesis	COX-2, VEGF, MMP− 2/− 7/− 9 ↓	HT-29	[69]

Table 1 (continued)

Compound	Reported effect—cellular process affected	Molecular mechanisms	Tested cell lines	References
SAMC	Cytostatic—G2/M arrest and finally apoptosis	Microtubule disarray JNK1, caspase 3 activity ↑	SW480 (IC_{50} = 150 µM)	[41]
		Microtubule disarray JNK1, caspase 3 activity ↑	SW480 (IC_{50} = 160 µM) HT-29 (IC_{50} = 175 µM)	[40]
		Microtubule disarray caspase 3 activity ↑ PARP cleavage ↑	Caco-2 SW480 SW620 (IC_{50} = 400 µM)	[42]
	Cytotoxic—apoptosis	TGF-β signaling, BAX, BAD ↑ caspase 9 activity ↑	SW620	[57]
Allicin	Cytostatic effect—cell cycle arrest	GSH ↓	HT-29	[43]
	Cytotoxic—apoptosis	STAT3 activity ↓ MCL-1, BCL-2, BCL-XL ↓	HCT116	[75]
		BAX ↑ BCL-2 ↓ translocation of Nrf2 to the nucleus ↑		[60]
Allicin + X-ray	Cytotoxic effects—apoptosis	NFκB, IKK ↓ IκBα ↑	HCT116	[59]
Z-ajoene	Cytostatic effect—G1/G0 arrest	β-Catenin, cMYC, cyclin D1 ↓ CK1α activity ↑	SW480	[44]
Thiacremonone	Cytotoxic effect—apoptosis	NFkB activity ↓ BCL-2, cIAP1/2, XIAP, iNOS, COX-2 ↓ BAX ↑ caspase 3 activity ↑ PARP cleavage ↑	SW620 (IC_{50} = 105 µg/ml) HCT116 (IC_{50} = 130 µg/ml)	[61]
MSeC	Cytotoxic effect—apoptosis	FAS, FASL, BAX ↑ ERK1/2, PI3K/AKT ↓ caspase 3/8 activity ↑ DFF45 and PARP cleavage ↑	COLO 205	[62]
DATTS/ DBTTS	Cytostatic/cytotoxic—apoptosis	Microtubule disarray	SW480 SW620 HT-29	[75]
DAS	Inhibition of migration and invasion	p38, PI3K, ERK, JNK, NFkB ↓ MMP− 2, − 7, − 9 ↓	COLO 205	[68]

*↑–upregulation/induction/activation, ↓–downregulation/suppression/inactivation

cancer cells, by causing DNA damage, DNA polymerase inhibition, oxidative stress or inhibiting the tubulin polymerization [30]. However, the existence of pure cytostatic agents is indicated, because when cytostasis occurs it will usually be followed by either cell death or cellular escape from stasis. Thus, it has been indicated that most of the anticancer agents are both cytostatic and cytotoxic and either property is dependent on the dose used, time point measured, phase of the cell cycle when the compound is administered and cellular context [31]. However, in this review, the biological activity of the various garlic-derived compounds and extracts will be considered cytostatic if it manifests as a cell cycle arrest in any of the cell cycle phases.

Garlic-derived individual compounds

One of the first studies that focused on the cytostatic activity of garlic-derived individual compounds on CRC cells was published in the year 2000, demonstrating the growth-inhibitory effect of diallyl disulfide (DADS), an organosulfur compound found in garlic extracts on HCT-15 human colon tumor cells [32]. DADS's activity was associated with a strong G2/M phase arrest in the cell cycle progression by inhibiting the CDK1 (cdc2/p34cdc2) kinase activity. The key mission of CDK1 is to form complexes with cyclin B to promote the G2/M transition and thus the assembly of the mitotic apparatus and chromosome alignment in mitosis [33]. The assessment of the underlying mechanism by which DADS suppresses CDK1 kinase activity showed that the

suppression did not result from direct interactions with the protein, but rather through changes in factors influencing the formation and conversion of the enzyme to its active form. The proposed possible explanation suggests that the ability of DADS to inhibit CDK1 kinase activation occurs because of decreased CDK1/cyclin B1 complex formation and modest CDK1 hyperphosphorylation, likely through suppression of CDC25C phosphatase activity [32]. Since then, several other research papers demonstrated that DADS has the capacity to inhibit CDK1/cyclin B complex formation and/or activation by modulating its upstream regulators, thus inducing a G2/M cell cycle arrest.

For example, after COLO 205 cells were treated with DADS, the CDK1/cyclin B complex was found to be decreased, whereas the proteins level of its negative upstream regulators, CHK1, WEE1 and CDC25C-ser-216, were increased. Furthermore, the expression of CDC25C phosphatase, one of the main activators of the CDK1/cyclin B complex, was found to be decreased again, proving that DADS induced a G2/M cell cycle arrest in a dose-dependent manner [34].

Another relevant upstream regulator of the CDK1/cyclin B complex involved in the G2/M transition is the cyclin kinase inhibitor p21 (WAF1/Cip1), a well-known potent CDK1 inhibitor. In this context, Liao et al. [35] also investigated the possible mechanism by which DADS induces a cell cycle arrest in the human colon cancer cell line SW480. The results indicated that DADS induces a G2/M cell cycle arrest through downregulation of cyclin B1 and upregulation of the CDK1-inhibitor p21. Furthermore, p53 and PCNA, two other essential proteins involved in the G2/M transition due to their role in the DNA damage repair mechanisms, were found to be downregulated as well.

At least two other studies proved that p21 expression is upregulated by DADS treatment, suggesting that its modulation is a key factor in promoting the cell cycle arrest in the G2/M phase by this garlic-derived organosulfur compound. First, in two human colon tumor cell lines, HT-29 and Caco-2, DADS-induced G2/M cell cycle arrest was associated with increased p21 expression, but also with histone H3 and H4 increased acetylation, most probably due to an inhibition of the histone deacetylase (HDAC) activity [36]. Histone acetylation hampers the transition into mitosis, because substantial histone deacetylation is needed for chromosome condensation. Secondly, Huang et al. [37] demonstrated that the expression of p21 is upregulated by DADS administration on human colon cancer cell line HT-29. Furthermore, the SSH technique, which enables the identification of rarely expressed, but specific, DADS-inducible genes, was applied to better understand the molecular mechanism involved in the antitumorigenic effects of DADS. The study revealed that 50 genes, including p21, were involved in the DADS G2/M cell cycle arrest [37].

DATS, another garlic-derived compound, also proved to have cytostatic effects in colorectal cancer cells. In cancer stem-like cells (CSCs), DATS significantly decreased the number of tumorspheres in a dose-dependent manner, whereas several CSC markers (CD133, CD44, ALDH1, OCT-4 and NANOG) were downregulated after treatment in SW480 and DLD-1 sphere-forming cells [38]. The inhibition of the Wnt/β-catenin pathway was found to be the main mechanism of action by which DATS inhibits CSC proliferation and cell cycle arrest in the G1/G0 phase. DATS triggers both an increase in GSK3β expression and a decrease of β-catenin protein, which in turn lead to a downregulation of several Wnt/β-catenin target genes that are involved in the G1/S transition, such as cyclin D1 and cMYC [38].

Another study showed that DATS induces a cell cycle arrest in the G2 phase as well in two additional cell lines, HCT-15 and DLD-1 [39]. DATS prevented the proliferation of these human colon cancer cell lines by oxidative modification of tubulin at Cys-12β and Cys-354β. The depolymerization of microtubules that followed led to the disruption of the microtubule network, inhibited the spindle formation, and thus increased the cell population in the G2 phase [39].

SAMC is another garlic-derived compound that has cytostatic activities in CRC cells, Evidence that SAMC significantly suppressed cell proliferation was found in multiple cell lines, including SW480, HT-29, Caco- 2 and SW620, with IC_{50} values ranging between 150 and 400 μM [40–42] Furthermore, its cytostatic activity was comparable in terms of the magnitude of effects with the antitumor activity of sulindac sulfone, a drug frequently used in CRC chemoprevention [40]. The main molecular mechanism of action of SAMC is based on its capacity to bind to tubulin [32–34], which triggers a rapid microtubule depolymerization, microtubule cytoskeleton disruption, centrosome fragmentation and Golgi dispersion in the cytoplasm of interphase cells, and it also interfered with spindle formation in mitotic cells [41]. Based on these cellular modifications, cells are arrested in the G2/M phase of the cell cycle, demonstrating the cytostatic effects of SAMC [40–42]. Furthermore, due to the microtubule disarray caused by SAMC, the induced cell cycle arrest is usually accompanied by apoptosis, most commonly by activating JNK1, which finally leads to caspase 3 activation [40, 41] and PARP1 cleavage [42].

Besides DADS, DATS and SAMC, at least two other organosulfur compounds isolated from garlic were tested for their cytostatic activity against CRC cells, namely, allicin and Z-ajoene. Among the first to test allicin for its antiproliferative effects were Hirsch et al. in 2000 [43]. The authors investigated the cytotoxicity of purified allicin against colon (HT-29) cancer cells, in comparison with that of a crude water extract of garlic. Allicin alone proved to have the same efficiency in inhibiting cancer cell growth as garlic extract containing an equivalent concentration of this compound,

suggesting that allicin itself is responsible for the antiproliferative effect of the extract. The antiproliferative effect of this compound may be attributed to its ability to deplete cellular GSH levels below a certain threshold level. Because only a slight elevation of GSSG was observed in the allicin-treated cells, it was suggested that the underlying mechanism of the decrease in intracellular GSH is based on the conjugate formation, and not oxidation. Despite a substantial transient decrease in GSH induced by allicin in HT-29 cells, no apoptotic cell death occurred at concentrations up to 30 µM, so most likely the growth-inhibitory effect resulted from cell cycle arrest.

Z-ajoene, a sulfur compound found in garlic, had an inhibitory effect on the Wnt/β-catenin signaling pathway. The Wnt/β-catenin pathway is essential in the G1/S transition, whereas aberrant signaling through Wnt may result in carcinogenesis. In this context, Z-ajoene was found to inhibit the levels of cytosolic and nuclear β-catenin in SW480 cells, and thus to inhibit protein expression of cMYC and cyclin D1. As the mRNA expression of β-catenin was not affected, it was suggested that the sulfur containing compound regulates β-catenin at a post-transcriptional level. Z-ajoene reduced intracellular levels of β-catenin through the amplification of β-catenin phosphorylation at Ser45 in a CK1α-dependent manner [44].

Garlic-derived whole extracts

Besides individual compounds isolated from garlic, such as DADS, allicin or Z-ajoene, several complex extracts obtained from garlic bulbs were also tested for their cytostatic activity on CRC cells. Therefore, suppression of DLD-1 cells proliferation was also possible by treatment with an aged garlic extract (AGE) produced by natural extraction. Fresh garlic cloves were subjected to extraction in a water–ethanol mixture solution for more than 10 months, at room temperature. Flow cytometric analysis was performed to determine whether the growth-inhibitory effects of AGE were correlated with cell cycle arrest or with apoptosis. After treatment with AGE for 2 days, neither cell cycle arrest at any phase nor increase in the sub-G1 population, an indicator of apoptosis, was found. Since AGE was not able to induce the expression of cleaved caspase 3 or the increase of the activity of caspase 3 in DLD-1 cells, it was suggested that the antiproliferative activity of AGE against DLD-1 cells was independent of cell cycle arrest or apoptotic cell death. The authors demonstrated for the first time that the mechanism underlying the suppressive effect of AGE in human colorectal cancer cell is based on the delayed cell cycle progression by downregulation of cyclin B1 and CDK1 expression via inactivation of NFkB, but not on the induction of apoptosis or cell cycle arrest [45].

The antiproliferative effects of aged black garlic extract (ABGE) were also investigated in HT-29 cells and the results revealed that it was mediated via blockage of the PI3K/AKT-dependent pathway. The extraction was carried out through mixing mashed aged black garlic in ethanol 95% for 24 h. After evaporation, the obtained residue was dissolved in 0.9% saline to prepare a solution at a concentration of 1 g/ml. ABGE modulated the PI3K/AKT signaling pathway in HT-29 cells through the upregulation of PTEN, the negative regulator of PI3K signaling, and the downregulation of AKT expression. Moreover, treatment with different concentrations of ABGE (20, 50 and 100 mg/ml) led to a dose-dependent induction of G0/G1 cell cycle arrest and a G2/M + S decrease [46] (Fig. 1).

Cytotoxic effects

Garlic contains many cytotoxic compounds capable of inducing cell death [47]. The investigation of active compounds from natural sources which have the ability to activate the apoptotic process is of great importance, as they can be used as complementary treatment of cancer, or they can become part of the conventional therapies. The cytotoxic activity of several compounds found in garlic, including DADS, DATS or allicin, and of different garlic whole extracts, has been demonstrated on several human colon cancer cell lines in vitro.

Garlic-derived individual compounds

DADS is one of the most studied compounds in terms of cytotoxic activity in colorectal cancer cells, among all garlic constituents. DADS treatment usually induces apoptosis, mainly through the intrinsic apoptotic pathways, which together lead to the release of different apoptosis inducing factors (AIF) from the mitochondria and thus to caspase activation. Among the most important initial molecular changes triggered by DADS in CRC cells, which ultimately lead to apoptosis, are intracellular calcium concentration increase, reactive oxygen species (ROS) accumulation and p53 activation.

The increase in intracellular calcium at certain concentrations is known to induce calcium-dependent DNA fragmentation, by triggering apoptosis. In HCT-15 cells, after treatment with 100 µM DADS for 3 h, an increase by 41% in the intracellular calcium was observed, in contrast to dipropyl disulfide (DPDS) treatment which caused only a 12% increase. Furthermore, a linear relationship between intracellular calcium concentration and DNA fragmentation was revealed [48]. Another study demonstrated a strong accumulation of Ca^{2+} (52% increase) in COLO 205 cells after treatment with 50 µM DADS [34]. The rapid increase of Ca^{2+} in a DADS concentration-dependent manner is

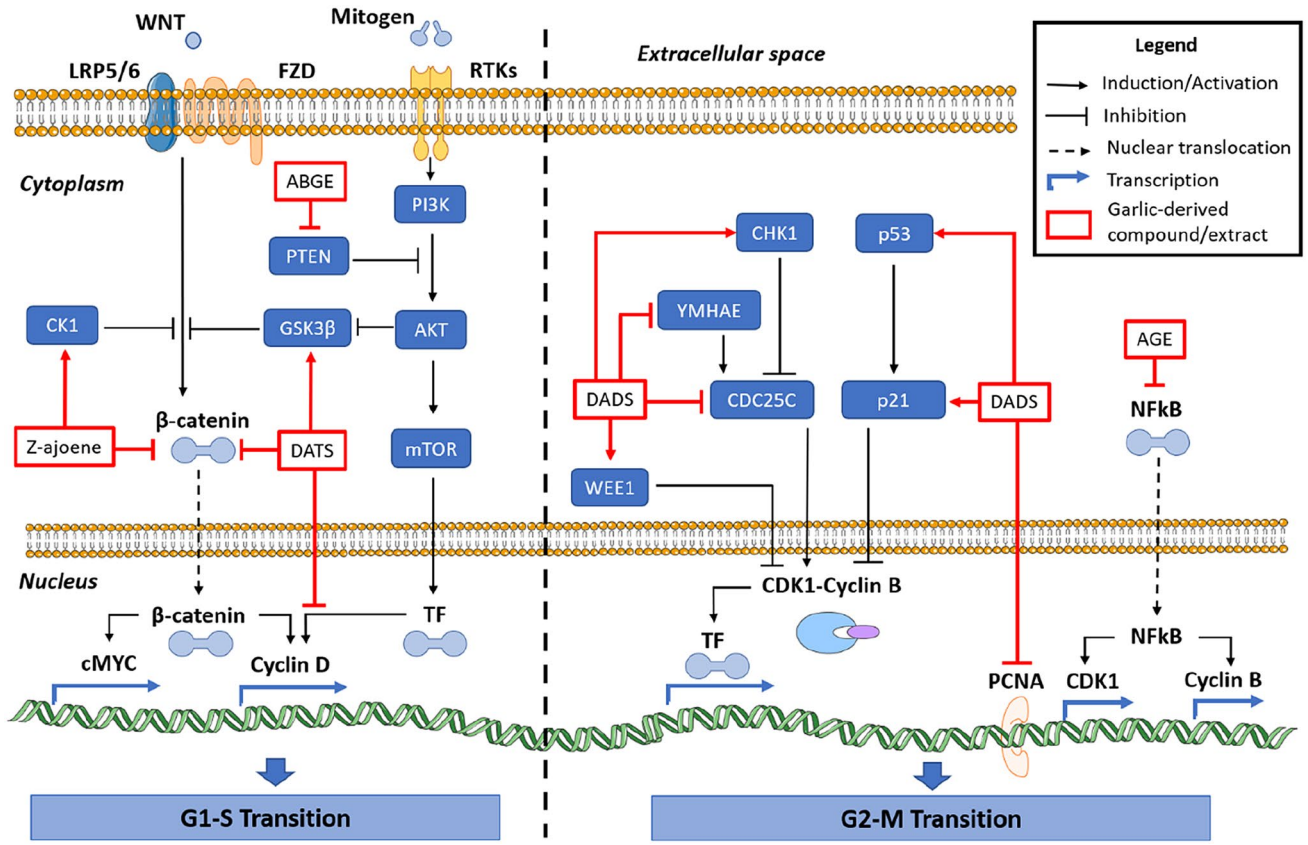

Fig. 1 Evidence-based molecular effects of different garlic-derived compounds and whole extracts in colorectal cancer cells that led to cell cycle arrest in either G1/G0 or G2/M phases

usually determined by phospholipase C-independent Ca^{2+} release from the endoplasmic reticulum (ER) and extracellular Ca^{2+} influx, at least in SW480 cell line [49]. Regardless of the process by which calcium accumulates intracellularly, it was confirmed that DADS induces apoptosis through this mechanism, rapid Ca^{2+} homeostasis disruption and Ca^{2+}-dependent generation of H_2O_2, resulting in at least caspase 3 activation [32].

Induction of apoptosis by DADS was also associated with ROS production in different CRC cells [34, 50]. To investigate the role of ROS in DADS-induced cell apoptosis, in one study, cells were pretreated with antioxidants (N-acetyl cysteine and reduced glutathione), and the resulting suppression of apoptosis demonstrated that ROS production plays an important role in DADS-mediated apoptosis. Interestingly, the same study also demonstrated that the process is accelerated if p53 is activated, which might be induced by the DNA damage caused by ROS [50].

The antitumorigenic, proapoptotic protein p53 also has an essential role in DADS-induced apoptosis, as several studies prove it is strongly expressed and activated by this compound. For example, after treatment of HCT116 cells with different concentrations of DADS (25–400 µM), an increase of p53 protein expression, in a dose-dependent manner, was observed [50]. The same effect was observed in COLO 205 cells as well, in which treatment with DADS increased the expression levels of p53 from 20 to 70% [34].

Regardless of the first molecular changes triggered by DADS treatment in CRC cells, the proapoptotic signals are usually integrated at the mitochondrial membranes, by strongly decreasing the membrane potential and thus releasing AIFs into the cytoplasm. The mitochondrial potential is tightly regulated by the pro- and antiapoptotic molecules from the BCL-2 family of proteins, several of which are modulated by DADS. For example, in COLO 205 cells, DADS treatment at a concentration of 50 µM decreased the mitochondrial membrane potential from 95% to 8.4%, by stimulating the expression of the proapoptotic molecules BAX and BAK and inhibiting the expression of the antiapoptotic molecular BCL-2 and BCL-XL [34]. These gene expression changes are triggered both by p53 and by the axis FAS–ASK1–JNK, both p53 and FAS being overexpressed in DADS-treated cells [34]. Furthermore, several other pro-survival molecules, that usually increase the expression of the antiapoptotic BCL-2 proteins, such as NFkB or COX-2, were shown to be inhibited by DADS, in a partially

GSK3β-dependent manner [51]. Another molecule that acts on the mitochondrial membrane permeability is the non-steroidal anti-inflammatory drug-activated gene (NAG-1), known to induce apoptosis through the intrinsic pathway. The NAG-1 protein level was also found to be strongly increased by DADS, in a p53-dependent manner [52].

Once the AIFs, such as cytochrome C, are released from the mitochondria, initiator caspase 9 is activated through proteolytic cleavage and in turn will activate effector caspases 3 and/or 7. Several studies demonstrated that both initiator and effector caspases, part of the intrinsic apoptotic pathway, are activated after DADS treatment in different CRC cell lines, in a time- and dose-dependent manner [34, 50, 53]. Furthermore, DADS-activated caspase 3 induces PARP cleavage, which in turn allows DNA fragmentation and, thus, apoptosis completion [50]. Interestingly, at least in the COLO 205 cell line, the initiator caspase for the extrinsic apoptotic pathway, caspase 8, was also found to be activated, suggesting that this mechanism might be induced in DADS-treated CRC cells as well [34].

To improve the treatment efficiency and increase the cytotoxic activity, DADS was also evaluated in combination with other molecules. For example, DADS was administered along with NaB (sodium butyrate), a short-chain fatty acid which is produced in the gut by bacterial fermentation of dietary polysaccharides, on HT-29 cells. Both DADS and NaB triggered early apoptosis events such as translocation of phosphatidylserine (PS) to the outer layer of the plasma membrane and induced late apoptotic events such as DNA fragmentation. Caspases 9 and 3 were activated by both separate treatments. However, the greatest effect in terms of caspase activation and apoptosis induction was observed in the combined treatment, with these compounds proving to have synergistic effects in CRC cells [53]. DADS was also evaluated as a sensitizer of CRC cells to apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) on four human colon cancer cell lines (HCT116, DLD-1, HT-29, and SW620) [54]. The results indicated that SW620 cells were the most resistant to TRAIL over a range of DADS concentrations, whereas DLD-1 cells were relatively sensitive to TRAIL apoptosis, the sensitivity of the cells depending on the BCL-2 basal expression. DADS sensitized CRC cells to TRAIL-induced cell death, mainly by downregulating BCL-2 expression [54].

Despite the significant cytotoxic activity of this compound, DADS has some major drawbacks too. Poor water solubility, lack of site specificity and low bioavailability are responsible for its limited clinical applications. To address these problems, DADS-loaded polymeric and pH-sensitive nanoparticles (NPs) for colon targeting were synthesized using a combination of Eudragit S100 (ES100) and poly-(lactic-co-glycolic acid) (PLGA). DADS-loaded ES100/PLGA-NPs proved to be more cytotoxic on CT26 murine colon carcinoma cells as compared to free DADS, the IC_{50} of DADS as a free drug and in targeted NPs being 1.32 ± 0.62 µg/ml and 0.14 ± 0.55 µg/ml, respectively. NPs were capable of releasing the therapeutics in the colonic region, at pH 7.4, making them suitable for further in vivo colon targeting [55].

DATS has also been proved to induce cell death in colorectal cancer cells. After exposure to 20 µM DATS, human primary colon cancer cells underwent remarkable nuclear changes, confirming that DATS induced apoptosis [56]. The proposed signaling pathway for DATS-induced apoptosis in human colorectal cancer cells involves lowering the mitochondrial membrane potential ($\Delta\Psi m$) via the changes in the BAX/BCL-2 ratio, which triggers the release of cytochrome C from mitochondria, and further the activation of caspase 9 and caspase 3, in both differentiated [56] and stem-like colorectal cancer cells [38].

The cytostatic activity of SAMC, which involved microtubule disarray and cell cycle arrest in the G2/M phase, is usually accompanied by apoptosis, most commonly by activating JNK1, as previously discussed [40–42]. However, to ascertain if SAMC induces apoptosis via other pathways as well, SW620 cells were treated with both MAPK inhibitors (including a JNK inhibitor) and SAMC. The results showed that if the MAPK signaling is inhibited in CRC cells, SAMC is capable of inducing apoptosis by activating the TGF-β signaling pathway, promoting the expression of BAX and BAD and finally activating the mitochondrial apoptosis pathway protein caspase 9 [57].

Besides cytostatic effects, allicin was found to possess cytotoxic effects as well. At least two research groups demonstrated that allicin acts on “rapid-acting" primary transcription factors such as STAT3 and NFκB, two regulators of several genes that control cell proliferation and cell survival [58, 59]. Allicin effectively induced apoptosis in HCT116 cells by decreasing the phosphorylated levels of STAT3 and thus downregulating MCL-1, BCL-2 and BCL-XL levels [60]. The overexpression of BAX was also demonstrated in allicin-treated CRC cells [60]. Furthermore, the combination therapy of low-dose allicin (10 µg/mL) with X-ray radiotherapy enhanced the growth-inhibitory effect, stimulated apoptosis and reduced the HCT116 colony-forming ability. The underlying molecular mechanism for this synergistic effect relies on the downregulation of both NFκB and IKK, an activator of NFκB, and upregulation of IκBα, one of the most important NFκB inhibitors [59]. Last but not least, allicin was tested to determine if it contributes to the activation of Nrf2 via its translocation to the nucleus [60]. Nrf2 is s a critical transcription factor that regulates the expression of over 1000 genes in the genome, both in normal and pathological conditions. The results showed that allicin induced significant nuclear accumulation of Nrf2 protein and, thus, emphasized that Nrf2 plays an important role in

the mechanism underlying the cytotoxic effects of allicin on several colon cancer cell lines [60].

Thiacremonone, a novel identified sulfur compound from garlic, was cytotoxic for SW620 and HCT116 cells in a dose-dependent manner, with IC_{50} values of 105 µg/ml and 130 µg/ml, respectively. Its main mechanism of action is based on the inhibition of NFκB, which in turn triggers a downregulation of the antiapoptotic genes BCL-2, cIAP1/2 and XIAP and inflammatory genes (iNOS and COX-2) and an upregulation of the proapoptotic gene BAX. Taken together, these molecular changes led to the proteolytic cleavage of caspase 3 and PARP, and thus to apoptosis [61].

Se-methyl-L-selenocysteine (MSeC) (250 µM) reduced cell viability in human COLO 205 cells to 24,8% after 24 h treatment. MSeC treatment induced cleavage of caspase 3, DNA fragmentation factor (DFF45) and PARP, proving apoptosis is the main mechanism of action. Interestingly, treatment with MSeC also triggered the upregulation of FAS and FASL, and further the activation of caspase 8, suggesting that apoptosis may occur mainly through the extrinsic apoptotic pathways, rather than through the intrinsic mechanism, as opposed to most of the other garlic-derived compounds. Furthermore, a downregulation of ERK1/2 and PI3K/AKT protein levels was found in MSeC cells that might contribute to the apoptosis initiation process [62] (Fig. 2).

Garlic-derived whole extracts

Besides individual compounds, whole garlic extracts were also tested over time for their cytotoxic activity on CRC cells. A crude garlic extract (CGE) decreased cell viability in a dose-dependent manner in COLO 205 cell line, whereas the DNA fragmentation electrophoresis analysis indicated that after treatment with 2 µg/ml of CGE, DNA fragmentation was induced [63]. The upregulation of BAX and concomitant downregulation of BCL-2 observed after treatment with CGE led to a decrease in the mitochondrial membrane potential, which caused an increase in active caspase 3 levels, suggesting the activation of the intrinsic apoptotic pathway. Another crude extract, obtained from crushed garlic cloves subjected to extraction in sterile water, was tested for its cytotoxic activity on Caco-2 cells [64]. The effects of the CGE extract were rather modest, only a 54% inhibitory effect on Caco-2 cell viability being registered at the highest dose tested (1 µg/mL). However, when Caco-2 cells were cocultured with mouse TIB-71 cells, which partially recreated a tumor-like xenograft microenvironment, the cell

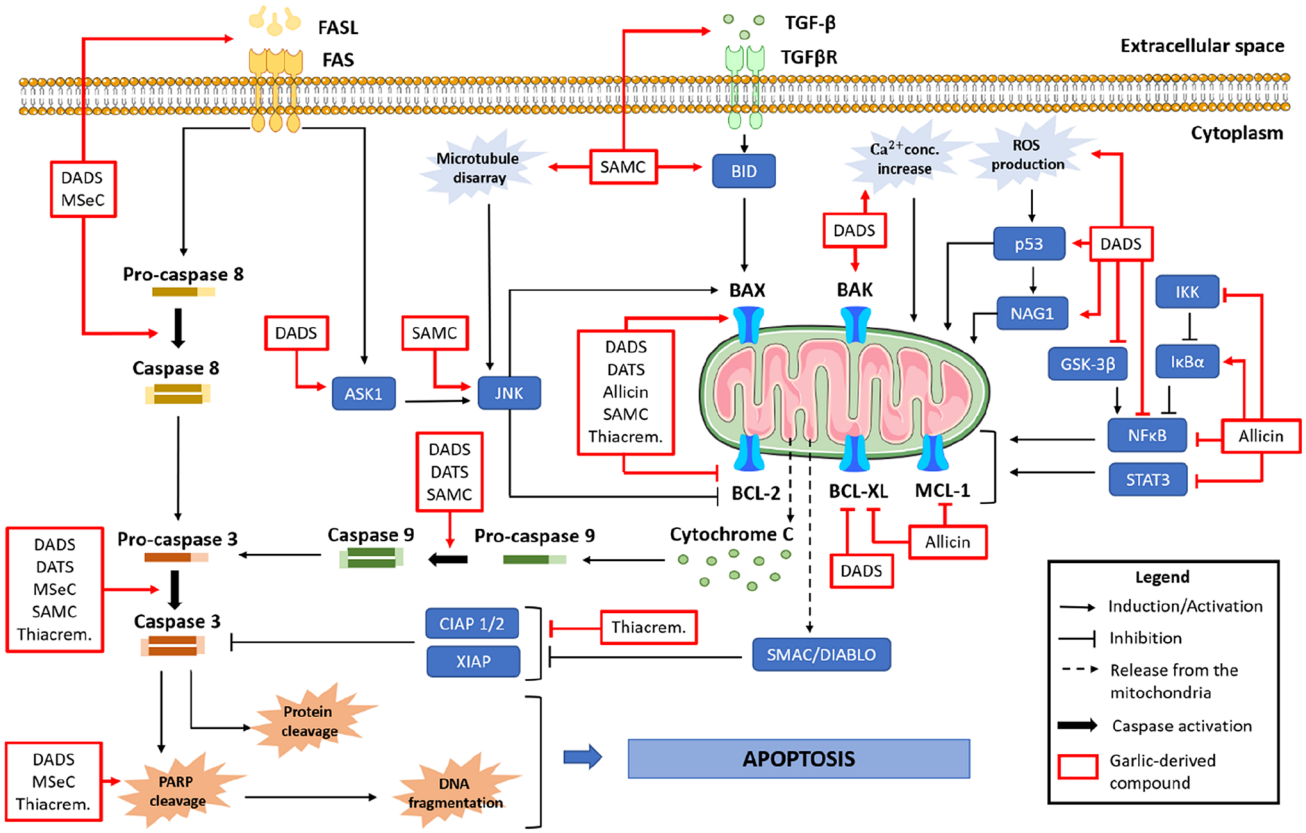

Fig. 2 Evidence-based molecular effects of different garlic-derived compounds in colorectal cancer cells that lead to apoptosis

viability strongly decreased at the same CGE concentration (60% after 24 h, 70% after 48 h and 90% after 72 h) [64].

A new lyophilized thiosulfinate-enriched garlic extract had cytotoxic effects as well, by inducing apoptosis on two human colon cancer cells, Caco-2, and HT-29. Purple garlic, after 1 month maturation, was extracted in ethanol 96% and acetone 99,5% in a stirred tank extractor. When tested in combination with two conventional drugs used in standard chemotherapeutic regimens for CRC patients, 5-fluorouracil (5-FU) or oxaliplatin, a stronger effect was obtained in comparison with 5-FU + oxaliplatin chemotherapy used alone. Analysis suggests that exposure to the combined treatment (thiosulfinate-enriched *Allium sativum* extract plus 5-FU/oxaliplatin) was able to reduce ≥ 20% cell viability. The inhibitory effect of this new garlic extract was accomplished at a concentration of 43–60 µg/mL allicin, equivalent to 260–360 µM [65].

The cytotoxic effects of synthetic allicin and garlic juice normalized to the allicin content against several mammalian cell lines, including human colon carcinoma HT-29, were compared in another study [66]. The effects between the two treatments were similar, both showing concentration-dependent trends on all tested cell line. HT-29 cells were sensitive to allicin or to allicin in garlic juice, showing a 50% reduction in the proportion of viable cells at 0.188–0.375 mM allicin. Interestingly, the authors suggest that the decrease in the proportion of viable cells is not due to apoptosis, but rather to other types of cell death, i.e., necrosis [66].

Most of the studies focused on extracts obtained from garlic bulbs, its roots being considered vegetable waste, and thus being unexplored from a scientific point of view. Therefore, Ahmed et al. [67] obtained an eco-friendly and medically efficient material by synthesizing silver nanoparticles using garlic roots. The ethanolic extract was obtained from air-dried, powdered roots by maceration and used to obtain green synthesized silver nanoparticles (TEEASR-based AgNPs). The first study on garlic roots showed that both total ethanolic extract of *A. sativum* roots (TEEASR) (IC_{50} values of 2.11 ± 0.03) and their green synthesized silver nanoparticles (AgNPs) (IC_{50} values of 0.47 ± 0.04 mg/mL) have a cytotoxic activity on Caco-2 colon cell lines, even stronger than doxorubicin, a well-known anticancer therapeutic agent. The cytotoxic property may be due to the interaction with the human inositol phosphate multikinase (HIPMK enzyme), as demonstrated by molecular docking analysis [67].

Inhibition of cell migration and invasion

Several organosulfur compounds such as DAS, DADS and DATS, as well as garlic whole extracts, proved to inhibit the migration and/or invasion of colon cancer cells.

In a comparative study, the effects of DAS, DADS and DATS on the migration and invasion of COLO 205 were investigated using a chemotactic directional migration assay in a 24-well transwell insert and Matrigel-coated transwell cell culture chambers [68]. Both migration and invasion were inhibited after treatment with either 10 or 25 µM of the compounds in the order of DATS < DADS < DAS. The expression levels of several proteins associated with migration, part of the ERK1/2, JNK1/2 and p38 signaling networks, were found to be decreased, which in turn led to the downregulation of MMP-2, MMP-7 and MMP-9 [68]. Matrix metalloproteinases (MMPs) are a group of enzymes that are responsible for the proteolysis of the basal membrane and extracellular matrix (ECM) proteins, their expression being correlated with the invasiveness of different cancer cells. The same MMPs were found to be downregulated after treatment with DATS, but this time in HT-29 cells [69]. Furthermore, the same upstream signaling proteins associated with migration and invasion, mainly ERK, JNK and p38, were found at lower levels after DATS treatment [69].

As invadopodia are required for cancer cells to migrate and invade nearby tissues, their regulatory gene, LIMK, has received much attention. The LIMK protein regulates actin polymerization via phosphorylation and inactivation of the actin binding factor ADF/cofilin, thus reorganizing the actin cytoskeleton and promoting tumor cell migration and invasion. Treatment with DADS (45 mg/l) on SW480 cells led to an inhibition of both gene expression and phosphorylation of LIMK1 protein, along with the modulation of several upstream and downstream proteins. Therefore, DADS suppresses cell migration and invasion based on the negative regulation of the RAC1–ROCK1/PAK1–LIMK1–ADF/cofilin signaling pathway [70].

An ethanolic AGE obtained by natural extraction from cloves was also tested in terms of its capacity to inhibit cell migration and invasion on three colorectal cancer cells HT-29, SW480, and SW620. The effect was different, depending on the type of cancer cell. AGE significantly inhibited the invasiveness of SW480 and SW620 cells, whereas no suppressive activity was observed on HT-29 cells [71].

Immunomodulatory effects

A limited number of articles focused on the immunomodulatory effects of garlic constituents in relation to colorectal cancer, but still some compounds were found to be capable of diminishing the immuno-suppressive environment during chronic inflammation in CRC.

Considering the role of inflammation in CRC, coculture of peripheral blood mononuclear cells (PBMCs) and colon cancer cell line SW48 was used for the first time to determine the impact of low molecular weight garlic proteins

(LMWGP) in cell proliferation, induction of regulatory *T* cells (Tregs) and cytokine secretion [72]. This garlic protein fraction was able to modulate several processes associated with inflammation in the tumor microenvironment. The number of pro-tumorigenic regulatory *T* cells (Tregs) was increased in coculture conditions, but treatment with LMWGP significantly depleted Treg cells. Proteins and cytokines known to help the tumor to evade the immune system, such as IL-10 and galactin-3, were found in lower concentration when LMWGP was administered. Thus, LMWGP might decrease the immuno-suppressive environment during chronic inflammation in CRC by helping the host to escape tumor-mediated immune suppression and to enhance antitumor immune response [72].

Allyl methyl disulfide (AMDS), another garlic-derived compound, was also investigated for the first time with respect to its immunomodulatory effects. At a concentration ranging between 20 and 150 µM, AMDS exhibited an inhibitory effect on the secretion of TNF-α-induced proinflammatory chemokines, IL-8 and IP-10 in HT-29 and Caco-2 cells. The mechanistic studies revealed that these properties are exerted through suppression of the IL-8 transcript and inhibition of IκBα degradation, which in turn hampers the translocation of NFκB p65 into the nucleus, resulting in the suppression of NFκB activation [73] (Tables 1 and 2).

In vivo studies

Garlic-derived compounds

The in vivo activity of several bioactive compounds from *Allium sativum* were investigated for their ability to inhibit CRC carcinogenesis and/or tumor progression in various animal models.

The antitumor activity of DAS, DADS and DATS was investigated in 36-week-old male BALB/c nude mice, previously injected with COLO 205 cells. DADS and DATS, but not DAS, significantly suppressed xenograft tumors (weight and size) in this animal model, DATS being the most cytotoxic among all tested compounds [76]. DATS has also been shown to be effective in mice bearing CT-26 allografts, in which a reduction of both tumor volume and weight was observed [77]. However, both DATS and DADS promoted the expression of several

Table 2 Main cellular processes affected by garlic-derived extracts in different colorectal cancer cell lines in vitro and their molecular mechanisms of action

Extract	Plant organ	Solvent	Extraction method	Reported effect—cellular process affected	Molecular mechanism	Tested cell lines	References
AGE	Bulbs	Water–ethanol mixture	Maceration + aging for 10 months	Cytostatic—delayed cell cycle	NFkB activity ↓ cyclin B1, CDK1 ↓	DLD-1	[45]
	Bulbs	Water–ethanol mixture	Maceration + aging for 10 months	Inhibition of angiogenesis	Endothelial cell motility, proliferation, and tube formation ↓	SW480 SW620	[71]
ABGE	Aged bulbs	Ethanol 95%	Maceration	Cytostatic—G0/G1 cell cycle arrest	PTEN ↑ AKT, 70S6K1 ↓	HT-29	[46]
CGE	–	Not specified	Not specified	Cytotoxic—apoptosis	BAX ↑ BCL-2 ↓ caspase 3 activity ↑	COLO 205	[63]
	Bulbs	Sterile water	Maceration (30 s)		caspase 3/7 ↑	Coculture of Caco-2 and TIB-71 cells	[64]
Thiosulfinate-enriched extract + 5-FU/oxaliplatin	–	Ethanol 96% and acetone 99,5%	Stirred tank extractor	Cytotoxic	Not specified	Caco-2 HT-29	[65]
TEEASR-based AgNPs	roots	Ethanol 95%	Maceration	Cytotoxic	HIPMK activity ↓	Caco-2 (IC_{50} = 0.47 mg/mL)	[67]

*Each extract is described in terms of the starting plant material, the solvent and the extraction method used ↑–upregulation/induction/activation, ↓–downregulation/suppression/inactivation

multidrug-resistance genes in vivo, such as MDR1, MRP1, MRP4 and MRP6, which must be taken into account in future studies [76].

DADS was also found to possess chemopreventive activity in CRC-induced animal models, mainly by suppressing inflammation. In a dinitrobenzenesulfonic acid (DNBS)-induced colitis mouse model, DADS was found to exert an anti-inflammatory effect by reducing the expression of pro-inflammatory cytokines such as IP-10 and IL-6 [78]. In this context, to assess whether the anti-inflammatory properties of DADS can lead to a decreased risk of developing colorectal cancer, Saud et al.[51] conducted a study in which FVB/N mice treated with azoxymethane/dextran sodium sulfate (AOM/DSS) were fed with a DADS supplemented diet. After dietary supplementation with 42 and 85 ppm DADS for 5 days starting 3 days post-DSS administration, a significant decrease in the total number of adenomatous polyps was observed. In the group treated with 85 ppm DADS, the expression of both GSK3β and NFkβ was inhibited by the compound, suggesting that DADS can prevent tumorigenesis by suppressing inflammation [51].

Based on the in vitro success of allicin on different CRC cell lines, its antitumor potential in vivo was investigated as well. In an AOM/DSS-induced CRC mouse model, allicin significantly reduced colorectal carcinogenesis, mainly by inhibiting the STAT3 signaling pathway [79]. The antitumor activity of allicin was also proven in BALB/c male mice bearing CT26 allografts, its administration causing a significant reduction of both tumor volume and weight [59, 79]. Furthermore, when allicin in low doses was administrated in combination with X-ray radiotherapy in the same experimental model, it enhanced the tumor radiosensitivity. Studies on their mechanism of action indicated that inhibition of NFκB signaling pathway may be responsible for this effect, as it was shown that NFκB is responsible for the tumor resistance to radiotherapy [59, 79].

Last but not least, the in vivo antitumor activity of SAMC was investigated in male athymic nude BALB/c mice bearing SW620-derived CRC tumors. The treatment with SAMC, administered orally for 16 days at a concentration of 300 mg/kg body weight, improved the general well-being of the mice, hampered tumor growth, and strongly suppressed metastasis, as no metastatic sites were observed in treated mice. Activated caspase 3 and cleaved PARP1 were detected in the treated cancer tissues suggesting that SAMC exerts its antitumor activity by regulating apoptosis [42]. Furthermore, SAMC used in combination with rapamycin, a drug used for the treatment of several cancers, suppressed tumor growth in mice with HCT-116 derived xenografts through enhanced autophagy, downregulation of p62 and activation of Nrf2 and its downstream gene NQO [62].

Garlic-derived whole extracts

The in vivo toxicity of an aqueous suspension of garlic (ASG) was investigated in Sprague–Dawley rats treated with AOM by orally administering 25 mg/day of garlic for 12 weeks after the first AOM injection. No toxicity, local or systemic, was observed, but instead, a significant reduction (42.1%) in the total number of aberrant crypt foci (ACF) following treatment was shown in all treated mice. Both cancer cell apoptosis and cell cycle arrest were triggered by the treatment, whereas the expression of the pro-tumorigenic protein COX-2 was found to be downregulated [80].

The antitumor properties of AGE were investigated on 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in Sprague–Dawley rats, suggesting its chemopreventive effects [81]. Given this premise, Jikihara et al. [45] evaluated the antitumor activity of AGE in male Fischer 344 rats in which carcinogenesis was also induced with DMH. When comparing the control rats with the ones fed a diet containing 3% AGE, both the total number of ACF and the mean tumor diameter were lower in the AGE-treated group by 28% and 32%, respectively. The results also showed that in the AGE-treated group, the labeling index (LI) of proliferating cell nuclear antigen (PCNA-LI) was significantly reduced in high-grade adenoma and adenocarcinoma cells as compared with the control group.

A boiled garlic powder (BGP), obtained by boiling the garlic bulbs for 15 min to inactivate alliinase and further homogenizing for 1 min using Polytron was also investigated for the antitumor potential in vivo. As shown in another study Chihara et al. [82] performed on rats treated with DMH and 5% BGP diet, a tendency for the numbers of ACF to decrease was observed, though the results were not statistically significant. Yet analysis of the mucin-depleted foci (MDF), a more recently discovered putative preneoplastic end point, showed a significant decrease in the numbers of MDF in treated rats, suggesting that BGP may be effective at least in the initiation stage. Also, compared to rats fed a basal diet, BGP treatment proved to be an effective protectant against O^6-MeG DNA adduct formation in the colorectum of rats fed 10% BGP [82] (Table 3).

Conclusions

Garlic-derived extracts and individual sulfur-containing phytoconstituents inhibit colorectal cancer development, mainly by promoting apoptosis and cell cycle arrest, and inhibiting cell invasion, angiogenesis and inflammation, both in in vitro and animal models. The molecular mechanisms for their antitumor effects are associated with the modulation of several well-known signaling pathways such as: PI3K/AKT, p38 MAPK, Wnt/β-catenin, NFκB, STAT3, ERK1/2,

Table 3 The in vivo antitumor, anti-inflammatory and chemopreventive activity of garlic-derived compounds and extracts

Extract/compound	In vivo activity	Animal models	Carcinogenesis initiation by	Doses (BW)/treatment time	Physiological effects	Mechanisms/genes involved/activation	Ref
DADS and DATS	Antitumoral effect	Balb/c$^{nu/nu}$ mice	s.c injection of COLO 205 cells (1×10^7/ mouse)	6 mg/kg, i.p. injected once every 4 days for 4 weeks	Weight and size of tumors suppressed	MDR1 gene ↑	[76]
DATS	Antitumoral effect	Balb/c mice	s.c. injection of CT26 cells (1×10^6/ mouse)	50 mg/kg, i.p. injected once every 4 days for 32 days	34% tumor growth inhibition	Apoptosis ↑	[77]
DAS and DADS	Anti-inflammatory effect	Swiss Albino mice	Intracolonic administration of dnbs	0.3–10 mg/kg, orally /2 days starting 24 h after DNBS administration	Body weight loss; reduced colon weight/colon length ratio; reduced signs of colon injury	IP-10 and IL-6 ↓	[78]
DADS	Anti-inflammatory effect	FVB/N mice	i.p injection with AOM, 10 mg/kg and 2% DSS after 10 days,	AIN-93G diet supplemented with 15, 30, and 60 mg/kg/daily/5 weeks/ starting 3 days after DSS administration	24.2%, 33.4% and 66.4% tumor burden reduction	GSK3β, NFkβ ↓	[51]
DAS and AMS	Antitumoral effect	Fischer 344 rats	AOM	50 mg/kg, orally/ 4 and 8 weeks	–	CYP2E1 ↓	[83]
SAMC	Antitumoral effect	Balb/c nude mice	s.c. injection of SW620 cells (3.5×10^6/ mouse)	300 mg/kg/day, gavage feeding/ 16 days	Necrosis and bleeding	Apoptosis: ↑ activation of capsase-3 and cleavage of PARP1	[42]
SAMC and SAMC + rapamycin	Antitumoral effect	Balb/c$^{nu/nu}$ mice	s.c. injection of HCT116 cells (1×10^7 per mouse)	SAMC: 300 mg/kg, oral gavage/daily/ 28 days	80.17% tumor growth inhibition	Autophagy: ↑ LC3-II, ↑ p62 ↓ Apoptosis: ↑ BAX, ↑ BCL-2 ↓ p53 ↑ Antioxidant capacity Nrf2 ↑	[84]
ASG	Chemopreventive effect	Sprague–Dawley rats	s.c. injections of AOM, 15 mg/kg	20 mg/rat/day, orally, continuously starting from 1st day of AOM injection	Total number of ACF: reduced by 45.27%; number of ACF with > 4 ACs: reduced by 44.11%	COX-2 ↓	[80]
AGE	Chemopreventive effect	Sprague–Dawley rats	s.c. injection of DMH, 20 mg/kg/ week /20 weeks	CE-2 diet supplemented with 4% AGE/daily/ 5 weeks	Total number of ACF reduced; number of ACF with 4 or more ACs reduced	Cell proliferation: ↓ MIB-5 ↓	[81]
AGE	Chemopreventive effect	Fischer 344 rats	s.c. injection of DMH, 20 mg/kg/ week/ 8 weeks	Diet supplemented with 3% AGE, one week after the last injection of DMH/ daily	Number of ACF: reduced; number of ACF with > 4 ACs: reduced; mean tumor diameter decreased	Cell proliferation: ↓ PCNA ↓	[45]

Table 3 (continued)

Extract/compound	In vivo activity	Animal models	Carcinogenesis initiation by	Doses (BW)/treatment time	Physiological effects	Mechanisms/genes involved/activation	Ref
BGP	Chemopreventive effect	Fischer 344 rats	s.c. injections of DMH, 40 mg/kg, on days 0 and 7 i.p. injections of DMH, 40 mg/kg/ week/ 5 weeks	Oriental MF diet supplemented with 1% and 5% BGP/8 weeks Oriental MF diet supplemented with 10% BGP/5 weeks	Total number of MDF: decreased by 41% and 65%	O^6-MeG DNA adduct levels ↓	[82]

*↑–Upregulation/induction/activation, ↓–downregulation/suppression/inactivation

CHK1/CDC25C/cyclin B1, as well as both the intrinsic and extrinsic apoptotic pathways. Furthermore, these organosulfur compounds are appealing as antitumor agents also due to their low general toxicity, as proven in several in vivo studies.

Although strong evidence for the antitumor potential of different garlic constituents against CRC is available, inconsistent clinical benefit was found for garlic consumption, as part of the human diet, in terms of its cancer preventive potential. This apparent contradiction between the in vitro/in vivo data and the observational studies is most likely due to the different dosage of garlic-derived compounds used/consumed among the two categories of studies. The concentrations used in in vitro/in vivo studies, especially when working with individual isolated compounds, are usually not reached within the normal human diet. Therefore, even if a garlic-based diet may not impact CRC initiation and promotion, its constituents might be good candidates for future conventional and/or complementary therapies.

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Data availability Data sharing is not applicable to this article as no new data were created in this study.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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