

# Significance of Garlic and Its Constituents in Cancer and Cardiovascular Disease

## Preclinical Perspectives on Garlic and Cancer<sup>1,2</sup>

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**ABSTRACT** Evidence continues to point to the anticancer properties of fresh garlic extracts, aged garlic, garlic oil, and a number of specific organosulfur compounds generated by processing garlic. These anticarcinogenic and antitumorigenic characteristics appear to arise through both dose- and temporal-related changes in a number of cellular events involved with the cancer process, including those involving drug metabolism, immunocompetence, cell cycle regulation, apoptosis, and angiogenesis. The ability of garlic and related allyl sulfur compounds to block tumors in the colon, lung, breast, and liver suggests general mechanisms that are not tissue specific. Whereas relatively few studies have compared the relative efficacy of water- and lipid-soluble allyl sulfur compounds, those that have when using chemically induced carcinogen models suggest little difference in response, whereas tumor proliferation/apoptosis is highly dependent on the species provided. A shift in sulfhydryl groups, alterations in glutathione:oxidized glutathione ratios, and resultant changes in cellular redox status may be involved in some of the phenotypic changes caused by allyl sulfur compounds. Such changes in thiols by allyl sulfurs may also account for the observed hyperphosphorylation of specific cell cycle proteins and the histone hyperacetylation that has been correlated with suppressed tumor cell proliferation. Whereas the anticarcinogenic and antitumorigenic data to date are impressive, additional studies are needed with more modest exposure to allyl sulfur compounds over prolonged periods. Likewise, additional studies are needed that incorporate transgenic and knockout models to assist in the identification of molecular targets for garlic and its associated allyl sulfur components. *J. Nutr.* 136: 827S–831S, 2006.

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Allium vegetables, including garlic, onions, leeks, chives, and scallions, are used throughout the world for their sensory characteristics as well as their apparent health benefits. The ability of these foods to serve as antimicrobial, antithrombotic, antitumor, hypolipidemic, antiarthritic, and hypoglycemic agents has kindled widespread interest in these vegetables as medicinal foods. Some of the most compelling evidence linking garlic and

related foods with activation against cancer comes from pre-clinical studies (1,2).

Although there is epidemiological support for their anticancer effects, the data are admittedly sparse (3,4). Cohort studies suggest an inverse association between garlic intake and the development of colorectal cancer; this also is possible at other cancer sites (3). Hsing et al. (4) reported that the reduced risk of prostate cancer in those consuming increasing quantities of allium vegetables was independent of body size, intake of other foods, as well as total calorie intake. Whereas data relating garlic intake to cancer risk are tantalizing, it is likely that variations in a variety of genetic and environmental factors may influence the response found among individuals.

It is becoming increasingly apparent that the response to specific foods or their components depends on the consumer's genetic background (nutrigenetic effects), DNA methylation and histone regulation (nutritional epigenomic effects), ability to induce or repress gene expression patterns (nutritional transcriptomics effects), occurrence and activity of specific proteins (nutriproteomic effects), and/or dose and temporal changes in cellular small-molecular-weight compounds (metabolomics effects) (5). Knowledge about each of these variables is needed to predict more accurately those individuals who will and will not respond to garlic or other allium foods. Expanding the information about similarities and differences in the -omic responses across the family of allium foods not only will provide valuable clues about specificity in response, but also will assist in the identification of surrogate fluids/tissues and associated

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biomarkers that can be used for predicting those who might be assisted or placed at risk by dietary intervention. Unraveling the importance of each of these potential sites of regulation is particularly challenging, but holds promise to help explain the mounting inconsistencies in the scientific literature about diet and cancer prevention interrelationships.

**Bioactive food components and their metabolites.** Whereas it remains to be determined which constituent or constituents within allium vegetables is most responsible for their proposed anticancer properties, there is a wealth of evidence suggesting that organosulfur constituents are most likely involved. The total sulfur content of garlic is known to reach 1% of its dry weight (6). Within the allium family, considerable variability occurs in the content and chemical sulfur species, which can be markedly influenced by the vegetation period examined (7). Thus, it is not surprising that considerable variability is observed when food disappearance data for this class of foods are correlated with cancer risk. The complexity of this interrelationship can also be further modified by constituents other than sulfur content and speciation (8). For example, the rather sizable oligosaccharide content may influence gastrointestinal flora or gastrointestinal function, both of which are associated with a cancer risk in some experimental models (9). Whereas garlic has a moderate amount of protein, it is a relatively rich source of the amino acid arginine, which has experimentally been reported to suppress inflammatory processes, which again has been linked to reduced cancer risk (10). The presence of several other factors, including selenium and flavonoids, may also influence several cellular processes that have been linked experimentally to cancer incidence and tumor behavior (11,12).

It is also unclear which intracellular metabolite may bring about a change in a specific cell signal and thereby lead to a phenotypic change. Whereas considerable information points to the ability of garlic to suppress the incidence and multiplicity of chemically induced tumors, it does not do so by changing the growth rate of the host. Thus, not all cells appear equally sensitive to the effects of garlic or its organosulfur constituents. Data from Koh et al. (13) indicated that low concentrations of diallyl disulfide (DADS)<sup>4</sup>, an organosulfur in processed garlic, were neuroprotective possibly by activating the phosphatidylinositol 3-kinase-dependent pathway (PI3K/Akt) and by inhibiting activation of glycogen synthase kinase-3 (GSK-3), cytochrome c release, caspase-3 activation, and poly(ADP-ribose) polymerase cleavage. However, cytotoxicity resulted from high exposures. Such data may indicate that the true cellular mediator is highly dependent on the intracellular concentration and thus argues that variation in response might be a manifestation of the uptake and/or the formation and removal rate of specific allyl sulfur intermediates. Differences in each of these variables may explain the observed reduced sensitivity of non-neoplastic cells, compared with neoplastic cells, to various allyl sulfur compounds (14).

Koh et al. (13) have also provided evidence that enhanced free radical formation may be involved in explaining the overall cellular response. In their studies with neuronal cells (nPC12), an increase in free radicals and membrane lipid peroxidation occurred when exposures reached 50  $\mu\text{mol/L}$  DADS that related to inhibition of cell growth. However, when exposures reached 100  $\mu\text{mol/L}$  DADS, cell death occurred, possibly as a result of the induction of apoptosis (13).

Not all allyl sulfur compounds appear equally toxic to cells. Water-soluble compounds found in deodorized garlic, such as S-allylcysteine (SAC), are far less toxic than the lipid-soluble compounds such as DADS (14,15). Whereas all cells will succumb to all allyl sulfur compounds if the concentrations become sufficiently high, there are clear differences among cells. More attention to how these sensitivities are related to the uptake, metabolism, and excretion of specific allyl sulfur compounds should help identify which tissues and cells will be most influenced by specific preparations of garlic that are in the marketplace.

**Free radicals, nutrigenetics, and garlic.** Reactive oxygen species (ROS) are known to arise from endogenous processes and from exogenous exposures. These ROS are believed to cause genetic oxidation and damage to DNA as well as other macromolecules. Unchecked, this oxidative damage may lead to a host of conditions, including cancer. Normally, this process is held in check by elaborate endogenous or exogenous antioxidant processes. Garlic is one of several foods with proposed antioxidant properties (1). It remains unclear whether a block in oxidation accounts for the plethora of published reports about the anticarcinogenic and antitumorigenic properties associated with garlic and its sulfur constituents. In fact, considerable evidence indicates that multiple molecular targets may be involved in determining the response to garlic.

One enzyme responsible for the production of ROS is myeloperoxidase (MPO). This enzyme occurs primarily in the primary granules of neutrophils and catalyzes the production of the potent bacteriotoxic oxidizing agent hypochlorous acid. It can also lead to the activation of a wide range of tobacco smoke mutagens and environmental pollutants to DNA-damaging metabolites, including those arising from aromatic amines, the promutagenic derivatives of polycyclic aromatic hydrocarbons, and heterocyclic amines (16–18). Gedik et al. (18) found that long-term administration of aqueous garlic extract (AE) alleviated liver fibrosis and oxidative damage as demonstrated by reduced MPO activity in rats with biliary obstruction. In another model, Sener (19) reported that peeled, crushed garlic extracts reversed decreases in glutathione (GSH) levels and increases in malondialdehyde levels and MPO activity caused by thermal stress. Thus, there is evidence that a change in free radicals may be one target for organosulfur compounds provided by garlic.

A single-nucleotide polymorphism (G463A substitution) occurs frequently in the promoter region of the MPO gene. The A variant allele confers lower transcriptional activation than the 463G (common) allele in vitro and the G allele has been associated with increased MPO mRNA and protein levels (16). Ahn et al. (17) reported that, when consumption of fruits and vegetables and specific dietary antioxidants was dichotomized at the median, inverse associations with either GA or AA genotypes were most pronounced among women who consumed higher amounts of total fruits and vegetables. Whereas the influence of polymorphic states in MPO A464G has not been evaluated as a determinant of the response to garlic, it certainly seems appropriate to do so. Regardless, supplemental garlic is known to influence the activities of several enzymes involved with regulating ROS, including superoxide dismutase, catalase, GSH peroxidase, glutathione S-transferase (GST), and glutathione reductase (20). The examination of the influence of polymorphisms in these enzymes may also help explain some of the observed variation in response to garlic in both preclinical and clinical studies.

**Epigenomics and garlic.** Epigenetic events represent another control site that can influence genetic expression. Several regulatory proteins, including DNA methyltransferases, methyl-cytosine guanine dinucleotide-binding proteins, histone-modifying enzymes, chromatin remodeling factors, and their

<sup>4</sup> Abbreviations used: AE, aqueous garlic extract; ARE, antioxidant responsive element; CYP2E1, cytochrome 2E1; DADS, diallyl disulfide; DAS, diallyl sulfide; DATS, diallyl trisulfide; DES diethylstilbestrol; GSH, glutathione; GST, glutathione S-transferase; GSK-3, glycogen synthase kinase-3; MPO, myeloperoxidase; NSAID, nonsteroidal anti-inflammatory drug; nr2, nuclear transcription factor 2; PI3K/Akt, phosphatidylinositol 3-kinase-dependent pathway; ROS, reactive oxygen species; SAC, S-allylcysteine; SAMC, S-allylmercaptocysteine.

multimolecular complexes are all involved in controlling the epigenetic process (21). Because epigenetic events can be influenced by several dietary components, they represent another plausible site for intervention with bioactive food components (22). Whereas the impact of garlic on DNA methylation has been woefully understudied, allyl sulfurs can influence DNA methylation processes indirectly by influencing carcinogen metabolism. The bioactivation of several carcinogens known to influence DNA methylation patterns (22) can be influenced by garlic and many of its sulfur constituents (23).

There is also evidence that some garlic constituents can influence another aspect of epigenomics, namely, histone homeostasis. Druesne et al. (24) reported that DADS and allylmercaptan effectively increased histone H3 acetylation in cultured Caco-2 and HT-29 cells. The histone H4 hyperacetylation was found to occur preferentially at lysine residues 12 and 16. The reason for this hyperacetylation may relate to the observed reduction in histone deacetylase activity (24). This change in hyperacetylation was also accompanied by an increase in p21(waf1/cip1) expression at mRNA and protein levels, again demonstrating that epigenomic events can influence subsequent gene expression patterns and lead to the accumulation of cells in the G2 phase of the cell cycle (24). DADS and allylmercaptan are rather unique in that they join relatively few food components, butyrate and sulforaphane, as modifiers of histone homeostasis (25).

**Garlic and transcriptomics.** Data from cDNA array studies reveal that the antiproliferative effects of DADS may relate to many changes in gene expression, including those related to alterations in cellular matrix gene expression (26). Specifically, DADS exposure down-regulated the expression of aggrecan 1, tenascin R, vitronectin, and cadherin 5, whereas DADS up-regulated 40S ribosomal protein stubarista protein (SA), platelet-derived growth factor-associated protein, and gliaderived neurite-promoting factor levels. These changes in matrix expression of protein may reflect a depression in cellular adhesion because this has been observed in other studies involving DADS. Franz et al. (27) reported that the increase in HT-29 cell detachment by aqueous garlic extracts related to an increase in epidermal growth factor receptor and integrin- $\alpha 6$  mRNA expression. Additional studies are needed to characterize more fully which changes in patterns of gene expression are critical to explaining the likely multiple targets involved with the anticancer and antitumorigenic properties attributed to garlic and its related sulfur constituents.

Andorfer et al. (28) compared in a short-term feeding study the effects of DADS, diallylthiosulfinate (allicin), and butylated hydroxyanisole on GST expression in the gastrointestinal tract and liver of mice. The effects of DADS and allicin on GST expression were especially prominent in the stomach and small intestine, where there were major coordinate changes in GST subunit profiles. In particular, the transcripts of the mGSTM1 and mGSTM4 genes, which share large segments of common 5'-flanking sequences, and their corresponding subunits were selectively induced. Whereas liver and colon GSTs were also increased, but to a lesser extent, there was no effect on heart, brain, and testis, suggesting that gene expression patterns are not equally influenced across all tissues. Their data also indicate these organosulfur compounds may operate on GST transcription through a reversible modification of certain protein sulfhydryl groups, shifts in reduced GSH:oxidized GSH ratios, and resultant changes in cellular redox status.

It is well known that allyl sulfur compounds can modulate drug metabolism systems, especially various phase II detoxifying enzymes. Studies by Chen et al. (29) demonstrated that the antioxidant responsive element (ARE) gene activation and

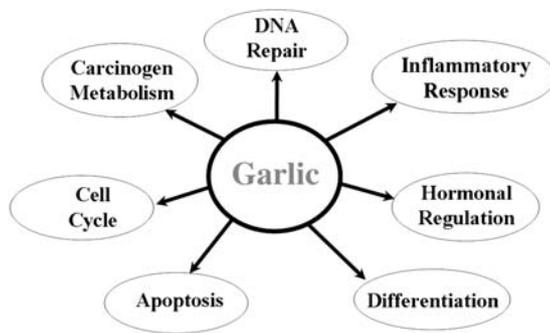
nuclear transcription factor 2 (nrf2) protein accumulation correlated with phase II gene expression induction. Using transient transfection HepG2 cells, they found that diallyl trisulfide (DATS)-induced ARE activity was inhibited by dominant-negative Nrf2 Kelch-like ECH-associating protein 1 and constructs. Likewise, treatment with thiol antioxidants decreased the ARE activity and Nrf2 protein level induced by DATS. Pretreatment with various upstream protein kinase inhibitors showed that the protein kinase C pathway was not directly involved in DATS-induced ARE activity, but instead the calcium-dependent signaling pathway appeared to play a role in the DATS-induced cytoprotective effect (29).

**Garlic and proteomics.** The examination of patterns of changes in protein expression and their modifications, or proteomics, presents a formidable challenge (30). Historical evidence has documented the marked effect that essential nutrient deficiencies can have on rates of protein synthesis and degradation. Today, it is clear that both essential and nonessential nutrients can not only influence protein anabolism and catabolism, but also markedly shift post-translation patterns by influencing phosphorylation, glycosylation, nitration, and ubiquitination. New information generated by a proteomics approach will undeniably have a major impact on understanding how subtle changes in diet can influence protein signals involved with cancer. Understanding proteomics will require greater attention not only to exposure to food components that are needed to bring about a change, but also to how this response is influenced by duration and compensatory cellular processes.

Numerous alterations in the occurrence and post-translation patterns of specific proteins have been observed following the ingestion of allium vegetables or their associated sulfur constituents. Whereas in many cases scientists have focused on the impact of allyl sulfur compounds on enzymatic activity, it is clear that activity may reflect a difference in the protein content as well as the amount occurring in the active state. For example, diallylsulfide (DAS) was found to induce the expression of wild-type p53 and down-regulates expression of mutant p53 in cells in culture when western-blot analysis and immunohistochemical protein detection were combined with multivariable flow cytometry (31). The increase in expression of the wild-type tumor suppressor gene protein p53 was accompanied by elevation of the levels of the cyclin-dependent kinase inhibitor p21/waf1.

Changes in activity may also be a result of a combination of the quantity and activity of specific cellular proteins. For example, exposure of synchronized colonic cells to DADS increased p34(cdc2) hyperphosphorylation by 15% (32). Consistent with its ability to slightly increase the quantity of hyperphosphorylated p34 (cdc2), DADS also decreased cdc25C protein expression. These findings suggest that the ability of DADS to inhibit p34(cdc2) kinase activation occurs because of decreased p34(cdc2)/cyclin B(1) complex formation as well as a shift in the p34(cdc2) hyperphosphorylation state (32). Allyl sulfides are not alone in their ability to bring about such changes because cyclin phosphorylation can also be modified by sulforaphane, a compound found in broccoli (33).

**Multiple targets.** Both water-soluble and lipid-soluble allyl sulfides can influence a number of molecular events involved with cancer (Fig. 1). These include inhibiting mutagenesis, blocking carcinogen DNA adduct formation, scavenging free radicals, as well as blocking cell proliferation, differentiation, and angiogenesis. Although there is a large body of evidence supporting each of these and other mechanisms, there is a need for additional research to demonstrate whether these changes are causally related to a cancer-preventive activity or not. Below is a brief account of some of the evidence linking garlic



**FIGURE 1** Dietary garlic may influence genetic and epigenetic events associated with several disease states, including cancer. Alterations in each of these processes are highly dependent on the form and quantity of allyl sulfur and the duration of exposure.

and related sulfur components with some of the processes linked to cancer.

**Carcinogen bioactivation.** Studies using a variety of chemical carcinogens indicate that the anticancer properties associated with garlic are not limited to a specific animal species or to a particular tissue and that both lipid- and water-soluble allyl sulfur compounds are effective. Because several different types of allyl sulfur compounds offer protection against chemical carcinogenesis, multiple mechanisms are possible (1,23,28,34). Certainly, it does not appear that a single mechanism could account for the observed protection based on the variety of carcinogens that have been examined. Nevertheless, a carcinogen class that appears to be particularly sensitive to blockage by water- and lipid-soluble allyl sulfurs is the nitrosamines by forming nitrosothiols (35). Their decrease in carcinogenicity may stem from an impediment in the formation and/or bioactivation of nitrosamines (34,35). A competitive block or autocatalysis of cytochrome 2E1 (CYP2E1), a member of the cytochrome P-450 superfamily, may account for part of this inhibition, at least for lipid-soluble organosulfur agents (34). DAS is sequentially converted to diallylsulfoxide and diallylsulfone by CYP2E1. Whereas polymorphisms in CYP2E1 might logically be assumed to influence the response to garlic, no such relation has been observed at least with the risk of esophageal and stomach cancer (36). Nevertheless, several studies have shown that a number of garlic compounds can reduce CYP2E1 activity presumably by serving as a competitive inhibitor. Because allyl sulfurs inhibit the actions of several carcinogens not requiring CYP2E1 activity, it is logical to assume that alterations in other phase I, or phase II, enzymatic targets may also account for protection (22,28,34,37).

**Hormonal regulation.** The association between estrogen exposure, either with or without progestin, and breast cancer risk continues to be a topic of immense interest and debate (38). Whereas no significant effects of garlic or its constituents on estradiol metabolism have been reported, a change in the biological response to diethylstilbestrol (DES), a synthetic estrogen known to increase mammary cancer in animal models, has been observed (39). Part of the effects of DES may stem from its ability to increase lipid hydroperoxides in mammary tissue. Recent studies demonstrate that this increase in ROS can be attenuated by providing DAS in the diet. This reduction was also related to a depression in DNA adducts (39).

The androgen dependence of the prostate gland, as well as some other tissues, is well established. Tissue culture studies provide evidence that several allyl sulfur compounds, in particular *S*-allylmercaptocysteine (SAMC), can enhance the rate of testosterone disappearance from the medium and presumably account for part of the antitumorogenic properties

of this agent (40). Collectively, SAMC treatment behaves similarly to androgen deprivation and thus provides clues that this effect may be mediated, at least in part, by the diminished effects of testosterone. Whereas it remains to be determined which mechanism accounts for these changes, it is conceivable that it involves the conversion of testosterone to metabolites that are less reactive with receptors (40).

**Inflammation and immunocompetence.** Part of the anticancer properties linked with garlic may arise from its ability to alter inflammation and subsequent immunocompetence. Leukocyte helper cells and inflammatory cytokine production have been reported to be reduced significantly in the presence of garlic extract (41). Studies by Lang et al. (42) suggest that allicin exerts an inhibitory immunomodulatory effect on intestinal epithelial cells and may thereby attenuate intestinal inflammation. Their studies revealed that allicin markedly inhibited the spontaneous and TNF- $\alpha$ -induced secretion of IL-1 $\beta$ , IL-8, IP-10, and monokine induced by INF- $\gamma$  (MIG) from the two different cell lines in a dose-dependent manner and suppressed the expression of IL-8 and IL-1 $\beta$  mRNA levels. In addition, allicin was found to suppress the degradation of I $\kappa$ B. However, the effect is complex because garlic derivatives appear to have both stimulatory (43) and inhibitory (44) properties in lymphocyte proliferation and LPS-induced TNF- $\alpha$  generation. Whether these variations in response relate to the type of sulfur compound tested, the duration of exposure, or some other modifier remains to be determined.

The nonsteroidal anti-inflammatory drug (NSAID)-activated gene (NAG-1) has been reported to possess both proapoptotic and antitumorogenic activities and is up-regulated by anticancer agents such as NSAIDs and, more recently, by DADS (45). Studies in vitro revealed that DADS treatment led to an induction of NAG-1 in a dose-dependent manner and that the induction of p53 preceded that of NAG-1. DADS did not induce NAG-1 or p53 in a p53 mutant cell line (45).

**Antiproliferation and apoptosis.** A variety of allyl sulfur compounds have been reported to reduce the growth rate of neoplastic cells in culture and in vivo (15,26,32,40). At least part of this reduced growth rate relates to a blockage in the cell cycle and most frequently in the G2/M phase. Most evidence points to the transitory nature of this inhibition, suggesting that the rate of clearance of allyl sulfur from cells is a determinant of the overall response. It is also clear that not all cells are equally susceptible to the deleterious effects of these sulfur compounds and, in particular, non-neoplastic cells tend to be less susceptible. As the concentration of the allyl sulfur compound increases, there is also a shift from depression in cell proliferation to greater involvement of apoptosis. This response may again relate to several changes with the cell as a consequence of an increase in oxidative stress caused by the various test compounds (46). Overall, the antiproliferative and apoptotic responses are dependent on the presence of the allyl molecule and the number of sulfur atoms. DADS is often observed to be >10 times more effective than DADS in retarding tumors. As reviewed previously, alterations in several molecular targets may explain the antiproliferative and apoptotic effects of allyl sulfur compounds (1,15,26,40,46).

As additional information about the specific targets for the various allyl sulfur compounds surfaces, it will be possible to develop better models for predicting those individuals who will benefit most from dietary change. This nutritional preemption approach should allow for the use of specific foods, such as garlic, at critical points that allow for a block in the initiation and progression of a pathway that leads to an unhealthy or lethal phenotype.

**Interaction with other food components.** Various food components may modify the ability of garlic to influence the

cancer process. Notable among these are the depression in response caused by variation in dietary sulfur amino acids, unsaturated fats, and selenium (47). In DNA carcinogen adduct studies, combining dietary garlic, selenite, and retinyl acetate was far superior to providing each ingredient individually. More recently, the effects of combining tomato and garlic were examined using a hamster buccal pouch carcinogenesis model (37). Combining tomato and garlic suppressed the incidence and mean tumor burden of hamster buccal pouch carcinomas that appeared to relate to a decrease in phase I enzyme and an increase in phase II enzyme activities. The effect of combining bioactive food components on the antitumorigenic properties of allyl sulfur compounds has not been adequately examined (15). However, similar to that observed with chemical carcinogenesis, there is evidence of a greater effect of allyl sulfur when combined with selenium than when provided alone.

As the era of molecular nutrition unfolds, a greater understanding about which of the many processes modified by garlic is critical to bringing about a phenotypic change. This information will be fundamental to the development of tailored strategies for reducing cancer burden. The identification of biomarkers that can be used to predict who will respond will be essential for effective intervention to occur.

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