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Review Article

Potential role of organic sulfur compounds from *Allium* in cancer prevention and therapy

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Abstract

The anticancer properties of fresh garlic extracts, aged garlic, garlic oil, and their specific organo-sulfur compounds of garlic. The anticarcinogenic and antitumorigenic characteristics appear through both dose and sequentially related changes in cellular events involved with the cancer process, including those involving drug metabolism, immune-competence, 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. Few studies have compared the relative efficacy of water and lipid soluble allyl sulfur compounds, when using chemically induced carcinogen models and suggested little difference in responses, 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 hyper acetylation that has correlated with suppressed tumor cell proliferation. Whereas the anticarcinogenic and antitumorigenic data are impressive and more studies are needed to exposures the allyl sulfur compounds anticancer activities.

1.Introduction

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 preclinical studies[1,2]. Although there is epidemiological support for their anticancer effects, the data are admittedly sparse[3,4]. An inverse association between garlic intake and the development of colorectal cancer; this also is possible at other cancer sites. 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. Garlic (*Allium sativum* L.*)* has a long history as being a food having a unique taste and odor along with some medicinal qualities. Phytochemicals present in the garlic have potential biological functionalities against several physiological processes[6]. Thus the therapeutic use and application of garlic for prevention of cancer or cardiovascular disease has widely been studied. Alk(en)yl sulfides are characteristic flavour component of garlic. The sulfides inhibit both initiation and promotion stages of tumorigenesis in experimental caricinogenesis model for various type of cancer. The allyl sulfides suppress cell growth and induce apoptosis in multiple cancer cell line[7,8]. However, the molecular mechanisms underlying the antitumorigenesis of allyl sulfide are still not fully understood. Some studies on the relationship between garlic consumption and incidence of cancers suggested that there is a reciprocal relationship between the two with respect to stomach and colorectal cancers[9]. The effect of allyl sulfides and molecular target of allyl sulfides is understand the anticancer mechanism elicited by the consumption of garlic. Number of sulfur atom in the allyl sulfides; the effect of diallyl monosulfide, diallyl disulfide, and diallyl trisulfide for their anticancer activity against human colon cancer cell lines, HCT-15 and DLD-1 cells. The changes in the cells are caused by diallyl trisulfide, which include the disruption of microtubule network formation and mitotic arrest as well as the induction of apoptosis. It was suggested that the modification of tubulin is a causative of the anti-cancer effect of diallyl trisulfide[10]. Skin cancer, the most prevalent cancer worldwide, can be broadly divided into melanoma and nonmelanoma skin cancer, depending on the cell type. The incidence of skin cancer has increased at an alarming rate in previous decades[11]. The WHO claims that one in three cancer cases are skin related, which stresses the global importance of skin cancer prevention. Basal cell carcinoma (BCC) is the most common type of keratinocyte tumor, exhibiting slow growth rate and spreading rarely[12]. In contrast, due to its rapid metastasis and chemotherapy resistance, melanoma is the least common formbut most lethal malignancy derived from melanocytes. Evidence suggests that excessive ultraviolet radiation (UVR) exposure, ozone depletion, genetic

and dietary factors, and lifestyle are associated with the development of skin cancer [13]. Skin cancer results from cocarcinogenic effects of different events, such as UVR induced DNA damage, oxidative stress, inflammation, and immune suppression [13,14]. Many natural dietary agents have received considerable attention because of their biological effects, such as antioxidant, anti-inflammatory, and anticarcinogenic functions [15,16].

2. 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, histonemodifying enzymes, chromatin remodeling factors, and their multimolecular complexes are all involved in controlling the epigenetic process[17]. Because epigenetic events can be influenced by several dietary components, they represent another plausible site for intervention with bioactive food components. 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[18] can be influenced by garlic and many of its sulfur constituents. Some garlic constituents can influence another aspect of epigenomics, namely, histone homeostasis. The 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. 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 [19]. DADS and allylmercaptan are rather unique in that they join relatively few food components, butyrate and sulforaphane, as modifiers of histone homeostasis[20]. 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[21]. Specifically, DADS exposure down-regulated the expression of aggrecan 1, tenascin R, vitronectin, and cadherin 5, whereas DADS upregulated 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. The increase in HT-29 cell detachment by aqueous garlic extracts is related to an increase in epidermal growth factor receptor and integrin-a6 mRNA expression[22]. 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. The effects of DADS, diallylthiosulfinate (allicin), and butylated hydroxyanisole on GST expression in the gastrointestinal tract and liver of mice[23], 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 59-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. The 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. Allyl sulfur compounds can modulate drug metabolism systems, especially various phase II detoxifying enzymes [24].

3. Potential Role of Organic Sulfur Compounds from *Allium* Species in Cancer Prevention and Therapy

Therapeutic agents are strongly supported by medicinal plants. Its beneficial effects on human health have been found, the identified active ingredients of plants to develop new drugs. Organic sulfur compounds (OSCs), especially allylsulfides, main compound class of Allium species (garlic, onion) responsible for anticancer and chemopreventive activities[25]. The potency of garlic and different OSCs ingredients were active against a huge set of diverse cancers derived from different tissues such as prostate, gastrointestinal tract, liver, breast, lung, skin, brain and blood[26]. The effects of the compounds are exerted by different mechanisms affecting multiple cellular pathways and being strongly based on interactions with cellular proteins, DNA or oxidative stressors[27] finally leading to an induction of cell death, e.g. via apoptosis or inhibition of proliferation. However mode of action of these OSCs is not yet fully understood and can be explained by their chemical reactivity, comprising both chemical bonds and other types of interactions with cellular contents, such as redox-reactions. Besides some organic sulfur compounds were shown to possess selective activity against cancer cells[27]. This is quite surprising due to the wide activity of the compounds and could be explained by different levels and patterns of protein expression, by different mechanisms or by different redox-levels of healthy and cancer cells. Overall properties of the compounds are quite favourable for ongoing drug development studies. Besides plants containing OSCs could be considered as functional food due to their beneficial effects on health. Skin cancer is a serious concern whose incidence is increasing at an alarming rate. Allyl sulfides-i.e., sulfur metabolites in garlic oil-have been demonstrated to have anticancer activity against several cancer types, although the mechanisms underlying these effects remain enigmatic. Our previous study showed that diallyl trisulfide (DATS) is more potent than mono- and disulfides against skin cancer. DATS inhibits cell growth of human melanoma A375 cells and basal cell carcinoma (BCC) cells by increasing the levels of intracellular reactive oxygen species (ROS) and DNA damage and by inducing G2/M arrest, endoplasmic reticulum (ER) stress, and mitochondria-mediated apoptosis, including the caspase-dependent and -independent pathways. This short review focuses on the molecular mechanisms of garlic-derived allyl sulfides on skin cancer prevention[28]. Alk(en)yl sulfides are characteristic flavor components of garlic. Several epidemiological study indicate that the risk of a certain cancer can be prevented by consumption of garlic. Anticancer property of garlic-derived alk(en)yl sulfides, and the molecular basis especially for diallyl trisulfide which is a major constituent of the garlic oil. Alk(en)yl sulfides with different numbers of sulfur atom (mono-, di-, and trisulfide) were synthesized and purified. The anticancer activity of the alk(en)yl sulfides was primarily examined using human colon cancer cells HCT-15 and DLD-1. The growth of the cells was significantly suppressed by diallyl trisulfide, but neither diallyl monosulfide nor diallyl disulfide showed such an effect. The number of cells arrested at G2/M phase, the cells with a sub-G1 DNA content, and the cells with caspase-3 activity were dramatically increased by diallyl trisulfide treatment. Diallyl trisulfide disrupted microtubule network formation of the cells, and microtubule fragments could be seen at the interphase. There was a specific oxidative modification of cysteine residues Cys12 beta and Cys354 beta, forming S-allylmercaptocysteines in the tubulin molecule. The diallyl trisulfide is responsible, at least in part, for the epidemiologically proven anticancer effect for garlic eaters [29].

The garlic-derived compound S-allylmercaptocysteine (SAMC) causes growth inhibition, mitotic arrest, and induction of apoptosis in

SW480 human colon cancer cells by inducing microtubule depolymerization and c-Jun NH(2) terminus kinase-1 activation. The effects of SAMC, garlic-derived and other organosulfur compounds. Among the 10 compounds tested, only SAMC, diallyl disulfide (DADS), and S-trityl-L-cysteine (trityl-cys) cause significant inhibition of cell growth with IC_{50} values of 150, 56, and 0.9 μmol/L, respectively. These three compounds also induce G(2)-M cell cycle arrest and apoptosis. The SAMC, the garlic-derived compound DADS exerts antiproliferative effects by binding directly to tubulin and disrupting the microtubule assembly, thus arresting cells in mitosis and triggering mitochondria-mediated signaling pathways that lead to apoptosis. The synthetic compound trityl-cys exerts its effect on M-phase arrest and growth inhibition by mechanisms that involve spindle impairment but do not involve disruption of microtubule structure or dynamics. Furthermore, trityl-cys does not induce marked loss of mitochondrial membrane potential or release of cytochrome c, but it does induce caspase-3 activation and poly(ADP-ribose) polymerase cleavage. Structure-function analysis suggests that both the allyl and the disulfide moieties are important features for the antiproliferative effects of SAMC and DADS. These findings may be useful in the identification, synthesis, and development of organosulfur compounds that have anticancer activity[30]. Allyl sulfur compounds are the major active constituents found in crushed garlic. Research has revealed that garlic and its lipid- or water-soluble components have many pharmacologic properties. Several studies investigated the influence of microwave or oven heating on the anticarcinogenesis property of garlic. [7,12-dimethylbenzene(a)anthracene (DMBA)] metabolites to rat mammary epithelial cell DNA. Allowing crushed garlic to "stand" for 10 min before microwave heating for 60 s prevented the total loss of anticarcinogenic activity. Study demonstrated that this blocking of the ability of garlic was consistent with inactivation of alliinase. These studies suggest that heating destroyed garlic's active allyl sulfur compound formation, which may relate to its anticancer properties [31].

Natural organosulfur compounds (OSCs) have been shown to have chemopreventive effects and to suppress the proliferation of tumor cells in vitro through the induction of apoptosis. The biochemical mechanisms underlying the antitumorigenic and anti-proliferative effects of garlic-derived OSCs are not fully understood. Several modes of action of these compounds have been proposed, and it seems likely that the rate of clearance of allyl sulfur groups from cells is a determinant of the overall response. The effects of natural allyl sulfur compounds on the cell detoxification system in normal and tumor cells. It has been already reported that several natural allyl sulfur compounds induce chemopreventive effects by affecting xenobiotic metabolizing enzymes and inducing their down-activation. Moreover, different effects of water- and oil-soluble allyl sulfur compounds on enzymes involved in the detoxification system of rat tissues have been observed. A direct interaction of the garlic allyl sulfur compounds with proteins involved in the detoxification system was studied in order to support the hypothesis that proteins possessing reactive thiol groups and that are involved in the detoxification system and in the cellular redox homeostasis, are likely the preferential targets of these compounds. The biochemical transformation of the OSCs in the cell and their adducts with thiol functional groups of these proteins, could be considered relevant events to uncover the anticancer properties of the allyl sulfur compounds. Although studies, using proteomic approaches and transgenic models, are needed to identify the molecular targets and modes of action of these natural compounds, the allyl sulfur compounds can represent potential ideal agents in anticancer therapy, either alone or in association with other antitumor drugs[32]. There is evidence that onions and garlic protect against cancer in humans. It has been suggested that this effect is due to the organosulfur compounds in these vegetables and that these substances act through induction of phase II detoxification enzymes. In the present studies, we have compared the ability of diallyl sulfide, dially disulfide, and diallyl trisulfide, compounds that are derived from garlic, to increase the activity of the phase II enzymes quinone reductase and glutathione transferase in a variety of rat tissues. We have also examined the onion-derived substances, dipropyl sulfide, dipropyl disulfide, dipropenyl sulfide, and dipropenyl disulfide, under identical conditions. Diallyl trisulfide and diallyl disulfide were potent inducers of the phase II enzymes. Dipropenyl disulfide was much less active, while little effect on enzyme activity was seen in animals dosed with dipropyl disulfide. Diallyl sulfide and dipropyl sulfide were weak inducers of quinone reductase and glutathione transferase, but dipropenyl sulfide was very active, with an effect similar to that of diallyl disulfide. It is possible that diallyl disulfide and diallyl trisulfide are important in the anticancer action of garlic, while dipropenyl sulfide could be involved in the beneficial action of onions [33].

4. Inhibitory action of garlic-derived allyl sulfides on chemical carcinogen-induced skin cancer in mice

Skin carcinogenesis is a multistage process involved in the alteration of the signaling molecules regulating cell proliferation, differentiation, and death activated by UV radiation or chemical carcinogens. These signaling molecules contain various transcription factors (e.g., p53, p21, activator protein-1 (AP-1)), cell cycle proteins (e.g., cyclins, cyclindependent kinases), antiapoptotic proteins, proapoptotic proteins, inflammatory enzymes, numerous protein kinases, Akt protein kinase), cell adhesion molecules, and growth factor signaling pathways. Therefore, agents that might prevent precancerous lesions caused by environmental carcinogens or that possess UV-blocking, antioxidant, antimutagenic, and anti-inflammatory properties may be useful against skin cancer[34]. The chemical carcinogen–induced two-stage skin tumor is a well-established animal model to study mechanisms of epithelial carcinogenesis. The initiation stage involving irreversible mutation in H-ras proto-oncogene is accomplished by the application of a carcinogen, such as 7,12-dimethyl benz(a)anthracene (DMBA). Subsequently, carcinogen-initiated skin is induced by repeated treatment with a tumor-promoting agent, such as 12-*O*-tetra decanoylphorbol-13-acetate (TPA) or phorbol myristate acetate (PMA), which causes the formation of benign tumors or papilloma. In this model, while the early stage of promotion is reversible, late-stage promotion and progression exhibit the irreversible phases of the tumorigenesis process. The chemopreventive effects of garlic oil against skin tumorigenesis initiated by DMBA and promoted by PMA**.** The efficacy of garlic-derived allyl sulfides are against chemical carcinogen-induced skin cancer in experimental rodents. The topical applications of garlic oil or allyl sulfides inhibit skin papilloma formation, reduce tumor incidence, and increase the survival rate in mice. Several mechanistic studies have been proposed to explain the antitumorgenesis effect of DAS, including modulating carcinogenmetabolism, inhibiting carcinogen-induced DNA damage, increasing cellular defenses system, and leading to apoptosis in carcinogen-induced skin tumor [35-37]. The DAS suppresses DMBA induced skin tumors through induction of apoptosis via modulation of ras-induced phosphatidylinositol 3-kinase (PI3K)/Akt, mitogen-activated protein kinase (MAPKs), and p53-mediated signaling pathways.30 Among the garlic-derived allyl compounds, DATS was more potent than DAS and DADS to suppress TPA-induced COX-2 expression [38]. The antitumor-promoting effect of DATS on TPA induced COX-2 and AP-1 expression is involved in modulation of JNK or Akt signaling on mouse skin carcinogenesis. Taken together, the prevention of carcinogenic progression by allyl sulfides has been attributed to its strong antioxidant, antiinflammatory, and antiproliferation properties. Allyl sulfides provide amultiprong beneficial approach for targeting multiple signaling pathways in skin cancer prevention.

5. Mechanistic studies of growth inhibition of allyl sulfides in skin cancer cells

The anticancer effect of allyl sulfides in prostate, lung, and colon cancers, chemoprevention of skin cancer by garlic organosulfur has recently received increased attention [35]. Extensive studies to elucidate the mechanism of DATS induced cell cycle arrest and apoptosis using human melanoma A375 cells and BCC cells as a model have been done[28]. A number of studies have indicated that the number of sulfur atoms on allyl sulfides determines their efficacy and biological activity, such as anticancer and anti-inflammatory activity. The ability of allyl sulfides to suppress the growth of cancer cells tightly correlates with the length of the sulfur chain. In line with previous reports, we revealed that DATS was more effective than DADS and DAS in decreasing cell viability of A375 and BCC cells. Moreover, DATS inhibited cell growth of A375 and BCC cells via activation of multiple target pathways[28]. The chemical properties and mechanisms determining the anticancer action of garlic-derived allyl sulfides have attracted recent scientific interest. Studies have shown that the antiproliferative effects of garlic-derived allyl sulfides are associated with their conversion to sulfane sulfur in tumor cells and/or to controlling proliferative signals[39]. For example, garlic organosulfur compounds bearing an *S*-allyl moiety can directly or indirectly target the redox-sensitive proteins at sulfhydryl sites, including cell-cycle checkpoint control proteins, apoptotic regulatory proteins, and transcription factors[30]. Results from findings and other studies are discussed in the following section to illustrate the mechanism of skin cancer prevention by garlic-derived allyl sulfides.

5.1 Induction of ROS generation and DNA damage in skin cancer cells

Human tumors frequently have defects in response to oxidative stress and DNA damage, compared with normal human cells. Hence, a DNA damage agent with less normal tissue toxicity is one of the newest cancer therapies [40]. The allyl disulfides can produce reactive oxygen species (ROS) directly by reactions relying upon the homolytic cleavage of disulfide bonds. Allyl sulfides can contribute to the generation of ROS, the depletion of glutathione (GSH), and the establishment of pro-oxidant conditions in cancer cells [41]. The differences between disulfides and trisulfides in the capability of ROS production and inability of monosulfides to form ROS can explain their diverse toxicities.

5.2 Inhibition of human melanoma metastasis *in vitro*

Epidemiological studies have identified that melanoma is a lethal type of skin cancer because of its relatively high probability to progress to metastasis and its resistance to chemotherapy. Therefore, it is imperative to develop efficacious preventive and therapeutic strategies for melanoma. The antimetastatic effect of garlic sulfur compounds. The result has shown that ajoene inhibits tumor cell growth *in vitro*, and strongly suppresses metastasis to lung in the B16/BL6 melanoma cell model in C57BL/6 mice. The DATS inhibits cell migration, adhesion, and invasion of A375 cells under noncytotoxic concentration as analyzed by wound healing assays and via a Matrigel invasion chamber system. The antimetastatic potency might be related to the decrease in the activity of matrix metalloproteinases (MMPs) induced by DATS, including MMP-2 and MMP-9. Similarly, a pervious study revealed that the antiadhesion effect of allyl sulfides involved in their sulfide group interacts with membrane lipids to modify the membrane fluidity or protein [42]. The garlic oil and allyl sulfides are effective in imparting protection against skin cancer. The mechanisms of DATS induced G2/M phase cell cycle arrest and apoptosis in skin cancer cells. Mechanisms underlying the skin cancer preventative effects of DATS are not completely understood, but known cellular responses to garlic-derived allyl sulfides include elevation of ROS and DNA damage, alteration of mitogenic and survival singling, and induction of G2/M arrest and apoptosis. Interestingly, we found that antioxidant NAC suppressed DATS-induced ROS production and growth inhibition. Although NAC nearly abolished DATS induced DNA damage, it only partially blocked the effect of DATS on growth suppression, indicating that the ROS-independent pathway is involved in DATS-induced cell death. Moreover, our study has shown that DATS-mediated G2/M arrest and apoptosis appeared to be selective for cancer cells, since normal HaCaT cells were resistant to growth inhibition by DATS [28]. This finding is consistent with previous studies in several cell and animal models [30]. In current research program, proteomic and genomic studies are in progress to further elucidate the different molecular events involved in skin cancer prevention by garlic oil and DATS in an animal model. The anticancer properties of fresh garlic extracts, aged garlic, garlic oil, and a number of specific organo-sulfur compounds generated by processing garlic. 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. 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 hyper phosphorylation of specific cell cycle proteins and the histone hyperacetylation that has been correlated with suppressed tumor cell proliferation [43]. Fresh garlic extracts, aged garlic, garlic oil and specific organosulfur compounds generated by processing garlic could alter carcinogen metabolism, inhibit tumor cell growth through induction of cell cycle arrest or apoptosis, or angiogenesis. The anticarcinogenic effects of certain garlic compounds may implicate at least in part a modulation of histone acetylation, a process involved in the regulation of gene expression, resulting from the inhibition of histone deacetylase activity. The sulfur compounds from garlic and histone acetylation and their potential for cancer prevention. The garlic compounds could inhibit histone deacetylase activity and induce histone hyperacetylation. The inhibitory effects of garlic organosulfur compounds might play a role in primary cancer prevention at doses achievable by human diet [44]. The garlic and its organic allyl sulfur components are effective inhibitors of the cancer process. The benefits of garlic are not limited to a specific species, to a particular tissue, or to a specific carcinogen. Several mechanisms are likely to account for this protection. Notable among these is a depression in nitrosamine formation and a reduction in carcinogen bioactivation. The benefits provided by garlic must be viewed as part of the entire diet, since several dietary constituents can influence the degree of protection. More than one compound is responsible for the anticancer properties associated with garlic. Future research should focus on how genetic variability and environmental factors influence the anticancer benefits attributed to garlic and its allyl sulfur components [9]. Diallyl disulfide (DADS), a sulfur compound derived from garlic, has been shown to have protective effects against colon carcinogenesis in several studies performed in rodent models. However, its molecular mechanism of action remains unclear. The anti-proliferative activity of DADS and to screen for differentially expressed genes induced by DADS in human colon cancer cells with the aim of exploring its possible anticancer mechanisms. The anti-proliferative capability of DADS in the HT-29 human colon cancer cells was analyzed by MTT assays and flow cytometry. The differences in gene expression between DADS-treated (experimental group) and untreated (control group) HT-29 cells were identified using two-directional suppression subtractive hybridization (SSH). Semi-quantitative reverse transcription polymerase chain reaction (semi-RT-PCR) was selected to confirm the results obtained by SSH. Based on the results, a dose- and time-dependent growth inhibition was observed in the DADS-treated HT-

29 cells. Forty-nine known genes and a new gene were found to be involved in the anti-proliferative effects of DADS by SSH analysis, and two cDNA libraries, DHDG and DHUG, containing both up- and down-regulated genes in colon tumor cells, were constructed. These genes were related to transduction, cell proliferation/growth/apoptosis and secreted/extracellular matrix proteins. Semi-RT-PCR results showed an expression pattern consistent with that of the SSH analysis. In conclusion, DADS showed anti-proliferative effects on colon cancer HT-29 cells, and DHDG and DHUG genes were found to be involved in this process. Further studies on the identification and description of these genes may allow a better understanding of the protective roles of DADS in colon carcinogenesis [45]. The use of non-conventional medicines, especially herbal medicine, is common in patients with cancers including haematologic malignancies. Diet components may also modify the risk of cancer through the influence on multiple processes, including DNA repair, cell proliferation and apoptosis. Garlic (*Allium sativum*), considered either food or herbal medicine, possesses antimutagenic and antiproliferative properties that can be used in anticancer interventions. We analyzed literature data on effects of garlic and garlic compounds which can serve as basic information to design clinical approach in oncohematology. Garlic contains water soluble and oil-soluble sulfur compounds. The anticancer effects exerted through multiple mechanisms such as: inhibition of metabolic carcinogenic activation, arrest of cell cycle, antioxidant and pro-apoptotic action. Evidence about the effects of main sulfur compounds diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), ajoene and S-allyl mercaptocysteine (SAMC) in oncohematology was described. Our research highlights that data on garlic in oncohematology are essentially represented by pre-clinical studies. Although these studies must be considered as preliminary, they provided insight into biological activities of garlic compounds and support a rationale for the use of substances such as DAS, DADS, DATS and ajoene as promising anticancer agents in oncohematology [46]. Garlic (Allium sativum L.) has a long history as being a food having a unique taste and odor along with some medicinal qualities. Modern scientific research has revealed that the wide variety of dietary and medicinal functions of garlic can be attributed to the sulfur compounds present in or generated from garlic. Although garlic produces more than 20 kinds of sulfide compounds from a few sulfur-containing amino acids, their functions are different from one another; e.g., allicin, methyl allyl trisulfide, and diallyl trisulfide have antibacterial, antithrombotic, and anticancer activities, respectively. The present paper reviews the physiological functions of garlic in the limited study fields of its antithrombotic and anticancer effects. Before describing these effects, however, we will discuss briefly some characteristics of garlic as a plant and some modes of absorption of orally-administered sulfur compounds from garlic [7]. Alk(en)yl sulfides are characteristic flavor components of garlic. The epidemiological study indicated that the risk of a certain cancer can be prevented by consumption of garlic. The anticancer property of garlic-derived alk(en)yl sulfides, and the molecular basis especially for diallyl trisulfide which is a major constituent of the garlic oil. Alk(en)yl sulfides with different numbers of sulfur atom (i.e., mono-, di-, and trisulfide) were synthesized and purified (>99%). The anticancer activity of the alk(en)yl sulfides was primarily examined using human colon cancer cells HCT-15 and DLD-1. The growth of the cells was significantly suppressed by diallyl trisulfide, but neither diallyl monosulfide nor diallyl disulfide showed such an effect. The number of cells arrested at G2/M phase, the cells with a sub-G1 DNA content, and the cells with caspase-3 activity were dramatically increased by diallyl trisulfide treatment. Diallyl trisulfide disrupted microtubule network formation of the cells, and microtubule fragments could be seen at the interphase. There was a specific oxidative modification of cysteine residues Cys12β and Cys354β, forming *S*-allylmercaptocysteines in the tubulin molecule. These results suggest that diallyl trisulfide is responsible, at least in part, for the epidemiologically proven anticancer effect for garlic eaters [29].

6. 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[47]. Within the allium family, considerable variability occurs in the content and chemical sulfur species, which can be markedly influenced by the vegetation period examined[48]. Thus, it is not surprising that considerable variability is observed when food disappearance data for this class of foods are correlated with cancer risk[49]. 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 [50]. 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[51]. 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[52]. 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[53] indicated that low concentrations of diallyl disulfide (DADS)4, 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[54]. The 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 mmol/L DADS that related to inhibition of cell growth. However, when exposures reached 100 mmol/L DADS, cell death occurred, possibly as a result of the induction of apoptosis [53]. 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 that the lipid-soluble compounds such as DADS[54].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.

6.1 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 anti-tumorigenic 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[55,56]. 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[56]. In another model[57], 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 [58]. 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 [55]. 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[17]. The influence of polymorphisms in these enzymes may also help explain some of the observed variation in response to garlic in clinical studies.

6.2 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 [59]. 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. Their decrease in carcinogenicity may stem from an impediment in the formation and/or bioactivation of nitrosamines. 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 [59]. 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 [60]. 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[61].

6.3 Antiproliferation and apoptosis

A variety of allyl sulfur compounds have been reduced the growth rate of neoplastic cells [62,63]. The reduced growth rate relates to a blockage in the cell cycle and most frequently in the G2/M phase. 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, 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 [64]. Overall, the antiproliferative and apopotic responses are dependent on the presence of the allyl molecule and the number of sulfur atoms. DATS is often observed to be .10 times more effective than DADS in retarding tumors. Alterations in several molecular targets may explain the antiproliferative and apopotic effects of allyl sulfur compounds [63,64]. 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 allowfor a block in the initiation and progression of a pathway that leads to an unhealthy or lethal phenotype.

6.4 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[65]. 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. 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 [66]. 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 antitumorigenic properties of this agent. 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 [63].

7. 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. 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[61]. 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[62]. 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.

8. Discussion

The effect of diallyl monosulfide, diallyl disulfide and diallyl trisulfide on the replication of human colon cancer cells. The reason why the diallyl trisulfide is more potent than disulfide or mono sulfide remains unclear. Because diallyl trisulfide is known to react with sulfhydryl group, modification of cysteine would be crucial for inhibiting cell growth. Diallyl trisulfide caused cell cycle arrest at G2/M phase. Immunofluorescence microscopy using anti-β-tubulin antibody clearly showed that diallyl trisulfide disrupted the microtubule network formation. Inhibition of the spindle formation by diallyl trisulfide, and the following cell cycle arrest at mitotic phase was also observed. The mitotic arrest caused by microtubule-interfering agents has been found to precede apoptotic cell death. Actually, the hypothesis that cell cycle arrest at mitosis is the primary signal to induce apoptosis has been widely accepted [67,68]. The diallyl trisulfide potently suppressed the growth of the xenograft without any obvious side effect. In summary, we demonstrated for the first time that a phytochemical derived from garlic, diallyl trisulfide, bound to specific cysteine residues in β-tubulin to form *S*allylmercaptocystein and that this could be the sole cause of cell cycle arrest and successive apoptosis with activation of caspase-3. The results suggested that diallyl trisulfide is responsible, at least in part, for the epidemiologically proven anticancer effect for garlic eaters. Skin cancer is a serious concern whose incidence is increasing at an alarming rate. Allyl sulfides-i.e., sulfur metabolites in garlic oil-have been demonstrated to have anticancer activity against several cancer types, although the mechanisms underlying these effects remain enigmatic. The diallyl trisulfide (DATS) is more potent than mono- and disulfides against skin cancer. DATS inhibits cell growth of human melanoma A375 cells and basal cell carcinoma (BCC) cells by increasing the levels of intracellular reactive oxygen species (ROS) and DNA damage and by inducing G2/M arrest, endoplasmic reticulum (ER) stress, and mitochondria-mediated apoptosis, including the caspase-dependent and -independent pathways. The molecular mechanisms of garlic-derived allyl sulfides on skin cancer prevention [28,69-72]. Epidemiological studies have suggested that the traditional Mediterranean diet containing high levels of antioxidants and phytochemicals has been associated with decreased incidence of skin cancer. Garlic (*Allium sativam L.*) is widely used in traditional herbal remedies and alternative medicine. The National Cancer Institute (NCI) set garlic on the top of a vegetable pyramid, representing potency in cancer prevention. The anticarcinogenic effect of garlic is attributed to the presence of organosulfur compounds such as allicin, allyl sulfides, ajoene, and *S*-allyl cysteine (SAC)**.** Fresh garlic cloves contain 0.2-0.5% garlic oil in the steam-distilled materials. Allyl sulfides in chemoprevention of skin cancer diallyl disulfide (DADS), diallyl trisulfide (DATS), and other allyl polysulfides are the most abundant compounds in garlic oil, accounting for nearly 94% of the total amount. The allyl sulfides suppress the growth of multiple cancer types in both *in vitro* and *in vivo* models [73-75]. Here, the review pertaining to anticancer properties of garlic oil and allyl sulfides against skin cancer, with special emphasis on the potential mechanisms.

9. Conclusions

Garlic-derived allyl sulfides possess an anticancer effect in several organs. The photopreventive effect of garlic-derived allyl sulfides is lacking. Studies showed that UV-induced photocarcinogenesis in the hairless mouse is widely accepted as a reliable preclinical animal model for the evaluation of chemoprevention agents. Therefore, the effects and mechanisms of allyl sulfides are in preventing photocarcinogenesis. The pharmacokinetics, bioavailability, and clinical investigations of diallyl trisulfide should be important for future recommendations for the practical application of garlic in the chemoprevention of skin cancer. 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.

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