1

Naturally Occurring Organic Sulfur Compounds: An Example of a Multitasking Class of Phytochemicals in Anti-Cancer Research

Claudia Cerella[1], Mareike Kelkel[1], Elodie Viry[1], Mario Dicato[1], Claus Jacob[2] and Marc Diederich[1]
[1]*Laboratoire de Biologie Moléculaire et Cellulaire du Cancer*
[2]*Division of Bioorganic Chemistry, School of Pharmacy, Saarland University, Saarbruecken,*
[1]*Luxembourg*
[2]*Germany*

1. Introduction

Allium plants, especially garlic (*Allium sativum*), have been cultivated since thousands of years all over the world not only as spicy food but also as medicinal plant. According to Block, the garlic plant was for the first time referred to in an Egyptian medical papyrus 1550 BC. In this Codex Ebers, 22 formulas of garlic were specified for the treatment of various disorders including heart problems, headache, bites, parasites and even tumors (Block, 1985). Today, the therapeutic value of garlic and other *Allium* vegetables is confirmed by multiple epidemiological and experimental studies. Especially prevention of cardiovascular diseases has been attributed to regular garlic consumption (Galeone et al., 2009; Kris-Etherton et al., 2002; Rahman & Lowe, 2006). Moreover, cholesterol lowering, hypoglycemic, immune-stimulatory, anti-microbial and even anti-cancer properties have been reported for garlic compounds (Agarwal, 1996; Amagase et al., 2001; Balkwill & Mantovani, 2001; Borrelli et al., 2007; Goncagul & Ayaz, 2010; Kalra et al., 2006). Epidemiological studies clearly show the correlation between moderate garlic intake and a low cancer incidence (Galeone et al., 2006; Kim, J.Y. & Kwon, 2009; Salem et al., 2011). A case-control study conducted in the 1980s in Italy revealed for example that people living in high-risk areas for gastric cancer consumed less garlic compared to people in low-risk regions where stomach and colon cancers were three times less frequent (Buiatti et al., 1989). The health beneficial effects of garlic and other *Allium* species make this plant family an extremely interesting research topic.

2. Bioactive chemicals and formulations from *Allium* vegetables

It appears that the biological activities of *Allium* plants are primarily attributed to organo-sulfur compounds (OSCs) (Bianchini & Vainio, 2001; Herman-Antosiewicz et al., 2007a; Jacob, 2006; Kalra et al., 2006; Powolny & Singh, 2008). Garlic and other *Allium* plants contain the highest amount of sulfur compounds described for common vegetables. In 1844, Wertheim provided evidence that OSCs are also causing the characteristic pungent garlic

odor (Lanzotti, 2006; Wertheim, 1844). Other pharmacologically interesting ingredients of *Allium* vegetables, on which we will not focus here, include sapogenins, saponins and flavonoids, the latter being mainly present in onion (Miean & Mohamed, 2001). Allixin and organo-selenium compounds also contribute to some biological effects (Corzo-Martinez M., 2007). It has been proposed that these non-sulfur compounds act together with the OSCs in a synergistic manner (Amagase, 2006). The composition of OSCs differs depending on the *Allium* species (Nencini et al., 2007), plant cultivation or storage conditions and processing methods (Verma S.K., 2008). Some OSCs are absent in the bulbs and require mechanical exposure like cutting, crushing or chewing to be formed. According to Verma et al., whole garlic bulbs contain 16 OSCs versus 23 OSCs after crushing (Verma S.K., 2008). In other reports 33 OSCs are reported in fresh garlic (Kalra et al., 2006). The cytoplasm of intact cloves contains biologically inactive γ-glutamylcysteine and S-alk(en)ylcysteinesulfoxides [(S-allylcysteinesulfoxide (alliin; 85%), S-methylcysteinesulfoxides (methiin; 10%) and S-trans-1-propenylcysteinesulfoxides (isoalliin; 5%) (Verma S.K., 2008)] that serve as precursors of volatile thiosulfinates (Kamel A., 2000; Lanzotti, 2006). γ-glutamylcysteine is hydrolysed and oxidized to S-alk(en)ylcysteinesulfoxides, mainly alliin (Corzo-Martinez M., 2007) or transformed into S-allylcysteine (SAC) by the action of γ-glutamyl transpeptidase. The latter reaction occurs in particular during wintering and sprouting of the garlic plant in order to ensure the production of sufficient alliin and isoalliin (Verma S.K., 2008). SAC can be oxidized to alliin (Kamel A., 2000; Lanzotti, 2006), which is then enzymatically transformed to diallylthiosulfinate (allicin) following the slicing of garlic cloves. This reaction is catalyzed by alliinase (also alliin lyase), an enzyme normally stored inside cytoplasmic microcompartments and released only after mechanical crushing (Weiner et al., 2009). Allylsulfenic acid, which is produced as short-lived intermediate, undergoes a spontaneous condensation reaction yielding allicin together with pyruvic acid and ammonium. Alliinase transforms cysteinesulfoxides to thiosulfinates in less than 60 seconds. The conversion of alliin to allicin is particularly rapid because enzyme and substrate appear in equal high amounts within the cell (Verma S.K., 2008). Due to their high instability, the volatile thiosulfinates are degraded within 24 hours into "second generation products" like oil-soluble mono-, di- and triallylsulfides (DAS, DADS, DATS) as well as vinyldithiins, thioacroleines and ajoene (Amagase, 2006; Kamel A., 2000; Munchberg et al., 2007), which still possess considerable biological activities and thus possibly represent the actual active compounds (Freeman F., 1995; Kamel A., 2000). Figure 1 summarizes the most important garlic-derived OSCs and their synthesis.

Higher polysulfides with four (DATTS) or more sulfur atoms are formed at higher temperatures (Lanzotti, 2006). According to data of Block reviewed by Kamel and Saleh, vinyldithiins are formed by dimerization of two thioacrolein molecules that arise from β-elimination of allicin whereas the generation of ajoenes is based on an initial S-thioallylation reaction of allicin (Block E., 1984; Kamel A., 2000). Further transformation leads to the formation of polysulfides that are present in multiple garlic preparations in substantial amounts and are comparatively stable. The most common allylsulfides are DAS, DADS, DATS and DATTS. Higher polysulfides up to heptasulfides are found less frequently and in low concentrations. Additional sulfur species can be generated from the interaction with intracellular thiols (cysteine, glutathione (GSH) or proteins) (Kalra et al., 2006; Weisberger & Pensky, 1958). Thus, S-allylmercaptocysteine (SAMC) arises from allicin whereas a similar reaction between DAS and cysteine gives rise to allylmercaptan (AM) (Kalra et al., 2006).

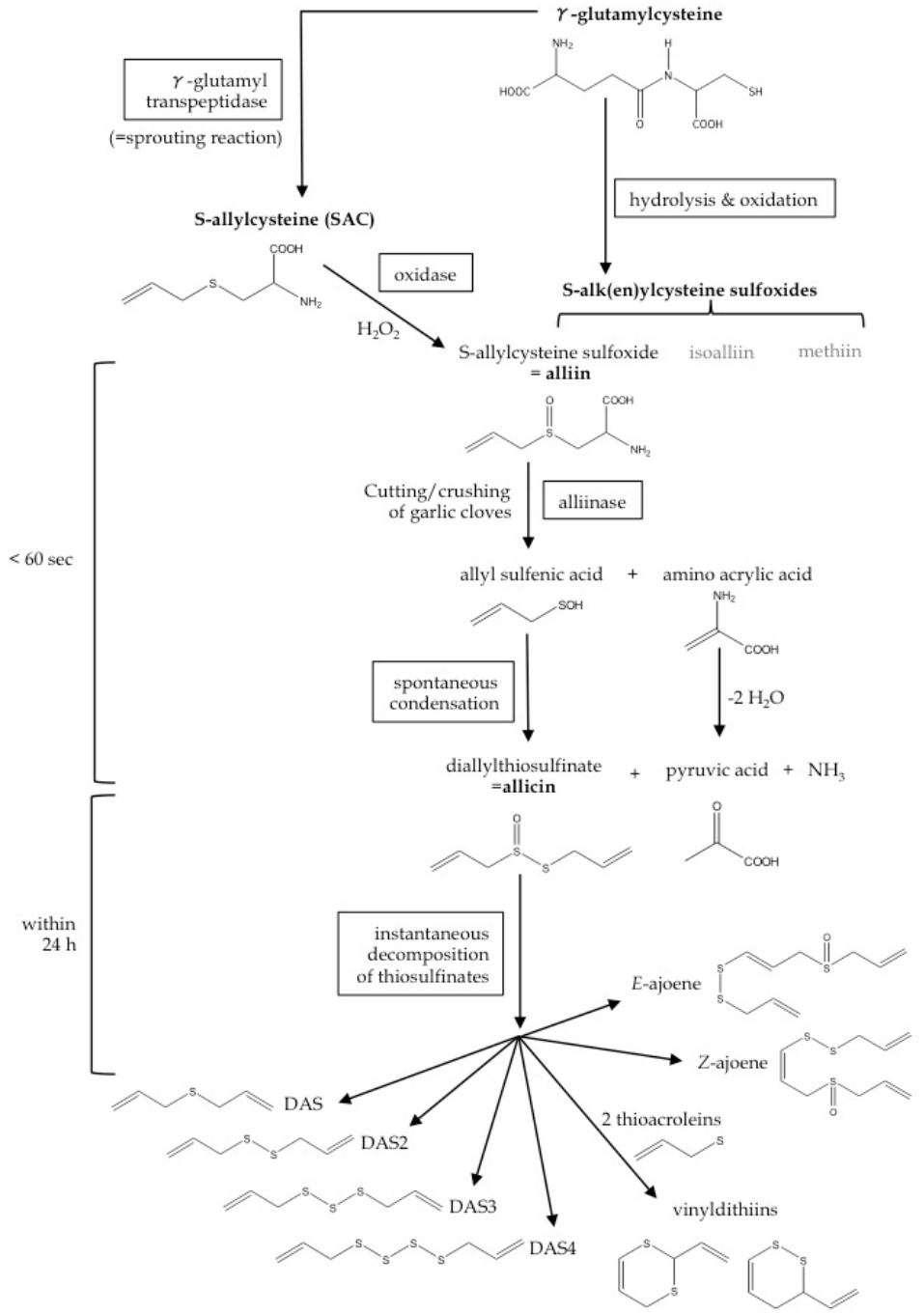

Fig. 1. Overview of the generation of different OSCs in garlic cloves

The different garlic formulations that are on the market vary significantly in their chemical composition. In view of clinical trials it is important to know about the components contained in diverse garlic preparations in order to correctly interpret the results. The most common garlic formulations include aqueous extract, garlic powder, garlic oil, oil macerate and aged garlic extract (AGE). For the aqueous extract garlic cloves are steeped in purified water. The primary compound in this extract is allicin (or alliin if alliinase is inactivated due to heating of the garlic extract). Further OSCs are allylmethylthiosulfonate, 1-propenylallylthiosulfonate and γ-glutamyl-S-alkylcysteine (Verma S.K., 2008). Garlic powder is produced by dehydration of crushed garlic cloves followed by pulverization. Thus, the composition of OSCs of the obtained powder is theoretically expected to correspond to the raw cloves. Nevertheless, the content of alliin (the major sulfur compound in raw and powdered garlic) and allicin varies considerably. Even if alliinase activity is comparable to fresh garlic, more than half of the alliin is lost during the dehydration process. Allicin is high in fresh garlic cloves, whereas the dehydrated powder is almost free of allicin, which might be explained by its instability (Amagase et al., 2001). Two types of garlic oil are generally synthesized: steam distilled oil or oil macerate. Garlic oil prepared by steam distillation of whole garlic cloves, ground in water, is completely free of hydrophilic OSCs and allicin (Amagase et al., 2001). According to Verma et al. it contains 57% diallylsulfides (DADS 26%, DATS 19%, DATTS 8% and lower concentrations of penta- and hexasulfides) in addition to 37% allylmethyl- and 6% dimethyl mono- to hexasulfides (Verma S.K., 2008). The available oil macerate products, consisting of mixtures of chopped garlic cloves homogenized and macerated in vegetable oil, are rich in allicin-derived OSCs including vinyldithiins, ajoenes and sulfides (Staba et al., 2001). Higher concentrations of dithiins and ajoenes are present in essential oil obtained by garlic extracted in organic solvents (Verma S.K., 2008). Another garlic preparation frequently used in *in vivo* studies or clinical trials is AGE. In contrast to aqueous extract, this extract prepared from sliced garlic cloves is aged for up to 20 months in a 15-20% ethanol solution. This aging process leads to a massive loss of allicin whereas water-soluble compounds like SAC and SAMC, which are odorless and stable, are enriched. AGE further contains some amounts of oil-soluble OSCs (Amagase et al., 2001).

3. Chemical properties of OSCs

3.1 Relevance of sulfur atoms for the reactivity of OSCs

The complex chemistry of garlic is believed to confer the plant anti-microbial activity as self-defense mechanism (Schneider, T. et al., 2011b). Chemical properties of the various OSCs strongly rely on the presence of highly reactive sulfur atoms, which exert multiple reactions including nucleophilic substitutions or redox reactions (Jacob, 2006; Munchberg et al., 2007). In line with the importance of sulfur, recent reports provided evidence that the biological activity of polysulfides directly correlates with their number of sulfur atoms, even if this relationship is not linear (Anwar et al., 2008). Thus, we found DATTS being the most active diallylsulfide in inducing apoptosis in U937 lymphoma cells, followed by DATS, whereas DAS and DADS instead were rather inactive (Cerella et al., 2009). Similarly, DATTS showed the highest antibiotic activity against *Staphylococcus aureus* (Tsao & Yin, 2001). The general trend seems to be that DAS is hardly active, DADS shows some activity, which is strongly increased in polysulfides with three or four sulfur atoms. Instead, five or more sulfur atoms

do not improve the reactivity of polysulfides significantly and the pentasulfide had for example effects on yeast comparable to DATTS (Jirousek L., 1956). Munchberg et al. explain these differences as follows. DADS is more active than DAS due to its oxidizing activity and the ability to generate thiols. The strong gain of activity of DAT(T)S might result from their reaction products, e.g. perthiols (RSSH). Even if perthiol formation is elevated for polysufides with four to six sulfur atoms, no further improvement of activity can be observed for the penta- and hexasulfides because of their declined stability (Munchberg et al., 2007).

3.2 Interaction with intracellular thiols

The most remarkable property of OSCs, especially reported for allicin and to some lesser extent for allylsulfides, is their reactivity towards intracellular thiols such as cysteine, GSH or proteins (Kalra et al., 2006; Weisberger & Pensky, 1958). These thiolation reactions give not only rise to the formation of further sulfur species, as mentioned above. More crucially, they lead to the inactivation of the affected proteins/enzymes (Weisberger & Pensky, 1958) and thus importantly interfere with cellular functions. Allicin is known to interact rapidly with thiol groups leading to the formation of S-allyl derivatives. Thus, the antibiotic activity of allicin has been suggested to be a consequence of its interaction with cysteine residues of different peptides and proteins of the target cell finally inducing cell death (Munchberg et al., 2007). Rabinkov et al. studied the chemical interaction between allicin and L-cysteine (as -SH carrier) and identified SAMC as the reaction product *via* RP-HPLC and subsequent ^{1}H and ^{13}C NMR analysis (Rabinkov et al., 1998). They further investigated the interaction of allicin with thiol-containing enzymes and found a very rapid inactivation of papain and two alcohol dehydrogenases, from which NAD^{+}-dependent alcohol dehydrogenase from horse liver was even irreversibly inactivated (Rabinkov et al., 1998).

An exchange reaction between a thiol (R'SH) and a polysulfide (RSxR) will lead to the formation of a mixed sulfide (RSxR') and a perthiol (RSSH) or other RSxH species (Munchberg et al., 2007). In the case of a tetrasulfide, two possible sites for a nucleophilic attack exist (at the central or the two terminal S-S bonds). Even if the formation of a trisulfide and perthiol seems to be the favored reaction, one cannot exclude that in addition disulfide (RSSR') and hydrogentrisulfide (RSSSH) generation takes place (Munchberg et al., 2007). For DATS, it is well documented that it targets specific cysteine residues within tubulin monomers (Hosono et al., 2008) thereby modulating tubulin conformation (Jordan et al., 1998) and consequently disturbing the microtubule (MT) network (Hosono et al., 2008). Hosono et al. suggested a mechanism by which DATS activates a thiol-disulfide exchange reaction through directly interacting with the thiol moiety of the cysteines C12 and C354 of β-tubulin. Finally, DATS binds covalently to tubulin *via* formation of SAMC-modifications (Hosono et al., 2005). A similar mode of action has also been reported for SAMC (Xiao et al., 2003). Li et al. likewise described an inhibitory effect of Z-ajoene on tubulin polymerization *in vitro* (Arora & Shukla, 2002). As it has been demonstrated that ajoene induces a rapid decrease of GSH (Scharfenberg et al., 1994), one may speculate that this OSC acts in a similar manner by directly targeting thiols. Such thiolation reactions on tubulin affect the formation of normal spindle microtubules during mitosis and thus trigger the induction of a cell cycle arrest (see Section 5.2). Accordingly, many studies showed that N-acetyl-cysteine (NAC), which is commonly used as antioxidant, counteracted the biological effects of OSCs (Wu,

X.J. et al., 2009; Xiao et al., 2005) (see Sections 5.2 and 5.5.3). The ability of NAC to increase the intracellular thiol pool has to be considered, which might quench the OSCs and thus prevent their interaction with thiol groups of potential target molecules. It is worth to mention that RSxH species arising from these thiolation reactions of polysulfides might also contribute to their biological activity as these hydrothiols are highly reactive and can for example act by reactive oxygen species (ROS) generation and as ligands for transition metal ions (Munchberg et al., 2007). Additional possible biochemical reactions related to polysulfides are reviewed by Munchberg 2007 in detail and include homolytic S-S cleavage, Sx transfer reactions and hydrophobic interactions with membranes and proteins (Munchberg et al., 2007). Antioxidant effects and metal binding ability are two further properties of OSCs that significantly contribute to their chemopreventive and chemotherapeutic activity and will be therefore discussed later on (see Section 5.3).

4. Chemopreventive/-therapeutic potentials of *Allium*-derived OSCs

4.1 Chemoprevention

4.1.1 Epidemiological studies

The endemic abundance of the *Allium* species contributes to their worldwide consumption and availability. The procedures to extract active natural occurring molecules or to synthetize them *de novo* in large amounts require relative low cost. Besides, the millenary use of these plants for dietary purposes ensures that the derived active natural compounds possess low or null systemic toxicity. All these considerations make the clinical exploitation of such derivatives/extracts of these plants an attractive and favorable strategy for chemopreventive and therapeutic purposes. Under this view, many epidemiological studies have been first conducted to scientifically validate the multi-beneficial effects on health (Scherer et al., 2010). Several interesting reviews give a critical overview of epidemiological studies examining correlations between the intake of *Allium* derivatives, in form of vegetables or supplements, and the chemoprotection from cardiovascular diseases, diabetes, cholesterol level alterations, gallstone formation and specific chronic inflammatory diseases. Here, we focus our attention on the studies dealing with the potential anti-cancer properties of *Allium* derivatives, taking into account the current lack of anti-cancer chemopreventive agents, on one hand, and the continuous demand of new targeted anti-cancer therapeutics, on the other hand.

From various case-control studies, it emerges that cancers affecting the digestive tract and the prostate appear as the most impacted ones. A number of studies investigated any inverse correlations between the consumption of *Allium* species and the incidence of cancers affecting the esophageal and stomach tract. These studies have been performed on geographical areas located in different continents as in Asia (China (Takezaki et al., 1999; You et al., 1989) and Japan (Gao et al., 1999)), Europe (Italy (Pelucchi et al., 2009) and Netherlands (Dorant et al., 1996)), or America (Venezuela (Munoz et al., 2001), Uruguay (De Stefani et al., 2001) and Hawaii (Hirohata & Kono, 1997)). Overall, a protective effect was reported, despite the obvious genetic variance existing among the populations examined in the different studies. Similarly, a reduced cancer risk has been widely documented in the instance of colorectal and prostate (Galeone et al., 2006; Hsing et al., 2002) (Fleischauer et al., 2000) forms of cancer. A limited number of studies explored the impact of a regular intake of

Allium vegetable on the incidence of cancers affecting breast, endometrium and lungs (Challier et al., 1998; Galeone et al., 2009; Satia et al., 2009). In many instances, inverse correlations have been also reported. Remarkably, amongst the human malignancies, gastric, colorectal and prostate cancers are the best characterized and documented ones for their multiple pre-neoplastic stages. This aspect provides additional tools to evaluate the impact of natural occurring compounds specifically on tumor progression. Accordingly, a regular consumption of garlic has been associated with the reduction in the incidence of pre-neoplastic lesions occurring in the gastric mucosa of individuals infected by *Helicobacter pylori* (You et al., 1998). In parallel, studies analyzing the preventive effect of garlic extracts on colorectal cancer have evidenced their suppressive potential on the development and progression of colorectal adenomas (Tanaka et al., 2004; Tanaka et al., 2006). A population-based study analyzing the impact of a diet rich in *Allium* vegetables on the incidence of prostate cancer showed that the anti-cancer effects were more pronounced in men presenting localized rather than advanced forms (Hsing et al., 2002), thus implying major effects of *Allium* derivatives on pre-neoplastic steps.

The many published epidemiological studies seem to encourage large-scale and long-term clinical trials with *Allium* derivatives. Meta-analyses, however, have not always supported the same conclusions. A representative example is a study published in 2009 (Kim, J.Y. & Kwon, 2009) and evaluating the impact of garlic intake on the risk of different forms of cancer. The study consisted in a critical systematic review of the publications appearing in Medline and EMBASE databases in the period 1955-2007 and matching some selected keywords. The criterion adopted for the selection of the studies to be considered as relevant was the satisfaction of the US Food and Drug Administration's evidence-based review system for scientific evaluation of health claim (http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodLabelingNutrition/ucm073332.htm). The conclusions pointed out the lack of evidence that may support an actual chemopreventive effect of garlic. Other critical reviews of epidemiological studies remark moderate evidence (Fleischauer & Arab, 2001). Overall, these investigations highlight all the limits frequently affecting epidemiological studies. Most of all, the bias due to the interview system frequently adopted to screen the population, which does not provide rigorous information about the actual amount of product/s intaken; the deep heterogeneity in the form of administration/consumption of the vegetable of interest (as food or as supplement; if as food, cooked or raw or in form of extract). Therefore, these contradictory analyses prompt to select further diversified approaches to validate the anti-cancer effects of *Allium* species.

4.1.2 Experimental studies

In this view, an important point is the *in vivo* experimental investigation of the effects of *Allium* vegetables. Mainly based on murine models, similar studies permit to gain detailed insights. First of all, it is possible to select and test specific purified compounds deriving from the *Allium* species and by this way to identify the most active natural occurring compounds. Second, we have at our disposal several *in vivo* models to mimic tumor progression or to evaluate the therapeutic impact on different transplanted cancers. Their exploitation is a fundamental part in the assessment of the mechanisms implicated in the anti-cancer effects and in the establishment of the efficaciousness of new anti-cancer agents as well.

Many lines of evidence show an ability of garlic extracts to prevent pre-neoplastic lesions of the gastrointestinal tract. Azoxymethane is a carcinogenic agent that induces the development of precancerous lesions in the colon consisting of aberrant crypt foci (ACF). The regular administration of garlic extracts to rats exposed to this tumor promoter is able to reduce the size and the number of ACF (Sengupta et al., 2003, 2004b). The chemopreventive effects are associated with an attenuation of early pre-neoplastic events (*i.e.*, the expression of cyclooxygenase 2 (Sengupta et al., 2004a); and a reduction in lipid peroxidation (Sengupta et al., 2003). Moreover, the histological analysis suggested the triggering of apoptosis (Sengupta et al., 2004b). Similar evidence has been accumulated in experimental *in vivo* models of oropharyngeal, gastric and skin carcinogenesis (Arora & Shukla, 2002; Balasenthil et al., 2002; Bhuvaneswari et al., 2004; Kalra et al., 2006; Prasad et al., 2008; Tanaka et al., 2004; Velmurugan et al., 2005). Garlic extracts or purified OSCs generally lead to the expression of markers of apoptosis and the reduction of the pre-neoplastic lesions. A similar pattern of modulations has been frequently reconfirmed in the instance of xenograft mice models of hepatocellular carcinoma (Zhang, Z.M. et al., 2007) and refractory forms of prostate cancer (Howard et al., 2008; Singh et al., 2008).

4.2 Therapeutic implications

Garlic extracts and isolated OSCs manifest a direct cytocidal activity. This point will be discussed in further detail in section 5.5. Here, we focus our attention on the chemoadjuvant properties of *Allium* derivatives. Preclinical studies have demonstrated the ability of garlic to modulate carcinogen metabolism, suggesting that its consumption could also influence drug intake. Interactions between garlic and drugs have been well described such as in the case of AIDS medication (e.g. interaction with saquinavir and darunavir metabolism) (Borrelli et al., 2007). Some pieces of evidence underline the ability of OSCs to chemosensitize cancer cells to chemotherapeutic treatment by modulation of cytochrome P450 isoforms. This, in turn, affects the pharmacokinetics of the corresponding drugs (see sections 6). Allium derived compounds have been reported to counteract the nuclear factor κB (NF-κB), which is frequently implicated in the resistance of cancer cells to chemotherapeutics. The modulation of both cellular targets may be implicated in the chemosensitization to the same treatment. Studies on docetaxel, a chemotherapeutic administrated to patients affected by hormonal or colon cancers, show that both mechanisms may be implicated in the ability of OSCs to modulate its activity (Ban et al., 2009; Cox et al., 2006; Howard et al., 2008). Cox *et al.*, however, have shown that co-administration of garlic and docetaxel did not significantly affect the drug disposition in breast cancer patients but a reduction of its clearance in specific cases cannot be excluded (Cox et al., 2006). Besides, two studies have shown that powder and AGE feeding to rats was able to prevent nephrotoxic and cardiotoxic side effects of both cisplatin and doxorubicin, two chemotherapeutic agents successfully used in cancer therapy (Alkreathy et al., 2010; Razo-Rodriguez et al., 2008). However, further investigations are needed to evaluate the actual potential interaction between garlic and chemotherapeutic agents. In addition, data suggested that immune-enhancing activity of garlic could take part in its anti-tumor effect. Indeed, garlic, and especially AGE, presented similar effectiveness to immunotherapy with bacillus Calmette-Guérin in transplanted bladder tumor mice. Garlic is able to promote the Th1 immune response by stimulating proliferation and tumor site

infiltration of macrophages and lymphocytes, increasing natural killers activity and enhancing the release of cytokines (e.g., IL-2, TNF-α, INF-γ) (Lamm & Riggs, 2001).

5. Mechanisms of chemopreventive/-therapeutic activities of *Allium*-derived OSCs

5.1 Modulation of detoxification of xenobiotics

5.1.1 Modulation of the metabolism of carcinogens

The effectiveness of a chemopreventive compound can be evaluated by its ability to interfere with different stages of carcinogenesis: initiation, promotion and progression (Surh, 2003). It was found that garlic and some of its constituents prevent tumor initiation by inhibiting the activation of pro-carcinogens and by stimulating their elimination (for recent reviews, see (Herman-Antosiewicz et al., 2007a; Iciek et al., 2009; Melino et al., 2011)). Indeed, the metabolizing-process of carcinogens comprises two phases: bioactivation and detoxification. The pro-carcinogens are activated by phase I enzymes through different types of reactions such as oxidation, hydroxylation, hydrolysis, cyclization. These reactions are generally catalyzed by the superfamily of cytochrome P450-dependent monooxygenases (CYP450). In the second step, the reactive carcinogens obtained are inactivated by phase II enzymes (e.g., glutathione *S*-transferases, glutathione peroxidases, UDP-glucuronyl transferases, quinone reductases). These detoxication reactions require the conjugation with endogenous substrates (e.g. glucuronic acid, glutathione, sulfate) to enable the excretion of inactive products (Sheweita & Tilmisany, 2003).

Several studies have contributed to elucidate the mechanisms by which garlic prevents chemical-induced cancer in animal models. Opposite effects have been reported on the modulation of CYP450 family members, both up- and downregulation depending on the isoenzymes. CYP450 2E1, which is responsible for the activation of small polar molecules such as acetaminophens, benzene and nitrosamines, is the isoenzyme most frequently reported to be induced by garlic. For example, dietary intake of garlic oil or powder leads to the inhibition of the hepatic microsomal CYP450 2E1 activity in mouse and rat, respectively (Park et al., 2002; Zeng et al., 2009). Moreover, garlic constituents, especially DAS and allylmethylsulfide (AMS), are effective in reducing the hepatic CYP450 2E1 protein level and activity (Davenport & Wargovich, 2005; Wargovich, 2006). Brady et al. have investigated the mechanisms of action of DAS and showed that this compound could directly act as a competitive inhibitor of CYP450 2E1 enzyme (Brady et al., 1988). Moreover, DAS could act indirectly through the formation of the oxidized products, diallylsulfoxide (DASO) and subsequently diallylsulfone ($DASO_2$), by CYP450 2E1 itself leading to the autocatalytic destruction of the enzyme (Brady et al., 1991; Jin & Baillie, 1997). Other CYP450 isoenzymes were modulated after garlic treatment. As example, DADS and DATS increased the transcript and the protein levels of CYP450 1A1/2, 2B1 and 2E1 in rats (Wu, C.C. et al., 2002). Other CYP450 isoenzymes seem to be implicated such as CYP450 2B1/2, 2B6, 2B10 and 3A11 (Davenport & Wargovich, 2005). Indeed, DAS is able to activate the constitutive androstane receptor (CAR), which is known to regulate the expression of those ones (Fisher et al., 2007; Sueyoshi et al., 2011). A consisting body of evidence was obtained through animal survey model but recently Ho et al. have confirmed the inhibitory effect of garlic on CYP450-metabolism in Fa2N-4 human hepatocytes. Exposure to garlic extract reduced the expression and the activity of CYP450 2C9 but exerted no effect on CYP450 3A4 (Ho et al., 2010).

The induction of phase II enzymes of the carcinogen metabolism by garlic was clearly described. Especially, many studies have reported that garlic treatment enhanced glutathione *S*-transferase (GSTs) activities. GSTs are important detoxifying enzymes, which stimulate the clearance of reactive compounds by conjugation with GSH. The group of Singh has shown that the chemopreventive effect of DADS, against benzo[a]pyrene-induced forestomach cancer in mice, was mainly mediated through the induction of the pi class mGSTP1-1 in liver and forestomach (Bose et al., 2002; Hu et al., 1996). However, different isoenzyme profiles could be obtained according to the target tissue considered. Thus, DADS administration can also upregulate the alpha and mu (mGSTM1, mGSTM4) classes in stomach and small intestine of mice fed with this compound whereas liver and colon GSTs were modulated to a lesser extent (Andorfer et al., 2004). More recently, Tsai and colleagues have shown that the induction of GSTPs at mRNA and protein levels in Clone 9 cells treated by DADS or DATS was dependent on JNK-AP-1 and ERK-AP-1 signaling pathways (Tsai et al., 2007; Tsai et al., 2005). In addition to GSTs upregulation, a study performed by Fukao et al. has shown that intraperitoneal administration of DADS and DATS in rats led to an increase of hepatic quinone reductase activity (Fukao et al., 2004). In 2004, Chen and coworkers have suggested that the activation of the antioxidant response element (ARE) and the increased level of the transcription nuclear E2-related factor 2 (Nrf2) were correlated with the induction of the detoxifying enzymes NAD(P)H :quinone oxidoreductase 1 (NQO1) and heme oxygenase 1 (HO1) in human hepatoma HepG2 cells treated with DAS, DADS or DATS (Chen, C. et al., 2004). The implication of Nrf2 in garlic chemopreventive effects has recently been confirmed (Fisher et al., 2007).

Finally, data concerning the modulation of phase II enzymes such as GSH peroxidase (GPX), superoxide dismutase (SOD), catalase, N-acetyltransferase are more disputed. For example, Singh et al. have reported that DATS induces GPX activity in the lung of A/J mice (Singh et al., 1997). Conversely, Chen et al. failed to show that DADS treatment modulates GPX or SOD activities (Chen, L. et al., 1999).

5.1.2 Modulation of the efflux of carcinogens

In addition to the modulation of carcinogen metabolism, OSCs can also influence the activity of transporters such as P-glycoprotein (P-gp), which allow the efflux of xenobiotics from the cells. This activity is particularly interesting to improve the cancer response to chemotherapies in the case of multidrug resistance phenotypes. Indeed, the treatment of leukemia K562 cells resistant to vinblastine (K562R) with a non-cytotoxic dose of DAS enhanced the cytotoxic activity of vinblastine as well as other *Vinca* alkaloids. The authors showed that DAS reduced the protein level of P-gp in K562R cells at a level comparable to non-resistant K562 (Arora et al., 2004). Such beneficial effect was also described for ajoene which improves the chemotherapy-induced apoptosis of cytarabine and fludarabine in human acute myeloid leukemia cells (Hassan, 2004).

5.2 Cell cycle arrest

One fundamental feature of carcinogenesis is the uncontrolled proliferation of tumor cells. Under physiological conditions, cell cycle regulation involves sophisticated control systems to ensure the precise sequence of the different phases and to obtain two identical daughter cells (Vermeulen et al., 2003). Cell cycle progression occurs mainly by the sequential action of

cyclin-dependent kinases (Cdks). Cdks are positively regulated by interaction with their regulatory subunits cyclins. On the contrary, association with specific inhibitors (e.g., p21, p27, p57) negatively regulates their activities. Moreover, phosphorylation-dephosphorylation events are implicated. Thus, cell division cycle 25 (CDC25) phosphatases act as negative regulators by phosphorylating specific tyrosine and threonine residues on Cdks.

Cell cycle arrest is triggered in response to cellular stress such as DNA damage or MT network alterations through the activation of the cell cycle checkpoints (Sancar et al., 2004). The G1/S checkpoint prevents the replication of damaged DNA whereas in G2/M it avoids the cell to trigger mitosis until the replication is correctly achieved. The activation of these checkpoints involves several signaling pathways such as p53, p38 MAPK, ataxia telangiectasia mutated (ATM)/ATM and Rad3-related (ATR) and checkpoint kinase (Chk)1/2.

Many studies have reported antiproliferative effects of garlic and OSCs in various cancer cell models, generally through the induction of cell cycle arrest in G2/M phase. In particular, detailed studies have been done on neuroblastoma (SH-SY5Y), prostate (PC-3, DU145) and colon (SW480) cancer cells (for a recent review see (Scherer et al., 2009)). The group of Milner was one of the first to highlight the importance of the modulation of Cdk1 activity in OSCs-induced cell cycle arrest. They have shown that DADS treatment of human colon HCT-115 cells led to G2/M phase arrest by suppressing Cdk1 activity (Knowles & Milner, 1998). DADS increased the expression of cyclin B1 but reduced the formation of the active cyclin B1/Cdk1 complex. The authors also reported the presence of inactive hyperphosphorylation of Cdk1, which seems to be related to the downregulation of CDC25C phosphatase (Knowles & Milner, 2000). Singh's team has extensively studied the molecular mechanisms involved the inhibition of CDC25C activity. On one hand, they have demonstrated that this inactivation was dependent on the phosphorylation of the serine residue S216 CDC25C, which is recognized as a binding site for the cytoplasmic protein 14-3-3 (Xiao et al., 2005). The activation of ATM/ATR and p38 MAPK signaling pathways, leading to Chk1 activation, were clearly involved in this process (Herman-Antosiewicz & Singh, 2005; Xiao et al., 2009b; Yuan et al., 2004). Indeed, Chk1 or ATR protein knockdown markedly attenuated the DATS-induced cell cycle arrest features in PC3 cells (Herman-Antosiewicz et al., 2007b). However, no clear evidence was provided about the importance of p53 status in ATM/ATR and Chk1/2 signaling pathway activation (Jo et al., 2008; Wang, H.C. et al., 2010a; Xiao et al., 2009b). On the other hand, Xiao and colleagues have reported a ROS-dependent destruction of CDC25C, which occurred independently of its phosphorylation. Implication of ROS generation, such as superoxide ($O2^{\bullet-}$) and hydrogen peroxide (H_2O_2), was confirmed by pretreatment in the presence of the antioxidant NAC, which significantly reduced the oxidation and the degradation of CDC25C in DU145 cells treated with DATS (Xiao et al., 2005). However, a recent study of the same group challenged the role played by CDC25C in the antiproliferative effect of DATS. Indeed, ectopic expression of CDC25C or the presence of its redox-insensitive mutant in DU145 cells failed to confer protection against DATS-induced G2/M phase arrest. This effect seems to be mainly related to differential kinetics of nuclear translocation between Cdk1 and cyclin B1 (Herman-Antosiewicz et al., 2010). Finally, a recent report suggested that both diallyl- and dipropyltetrasulfides (DPTTS) could act as irreversible inhibitors of CDC25C (IC_{50} about 1μM) (Viry et al., 2011).

Other mechanisms of action involved in OSCs-mediated cell cycle arrest have also been identified. In particular, Hosono et al. have demonstrated, through the use of an *in vitro* cell-free model, that DATS inhibited tubulin polymerization. Mass spectrometry analysis has demonstrated that DATS induced the oxidation of two cysteine residues in the β-tubulin (C12 and C354) (Hosono et al., 2005). These results were confirmed in another study by the use of reducing agent (2-mercaptoethanol and dithiothreitol), which abolished the MT-disrupting activity of allicin (Prager-Khoutorsky et al., 2007). In addition, it was also reported that OSCs could modulate the transcription of important regulators of the cell cycle. As example, the DADS-induced G2/M phase arrest of colon cancer Caco-2 and HT-29 cells was associated with the upregulation of p21, which appeared to be closely dependent on the acetylation status of its promoter (see section 5.4) (Druesne et al., 2004). Figure 2 summarizes the modulatory effects of OSCs on cell cycle.

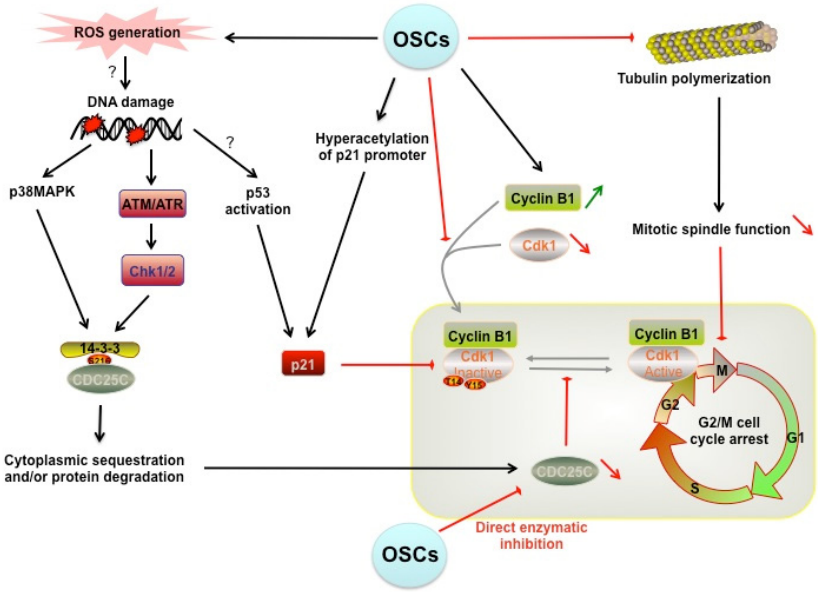

Fig. 2. Schematic overview of cellular targets of OSCs implicated in cell cycle arrest.

5.3 Redox modulation

5.3.1 Antioxidant activity

Cellular damage by oxidation of macromolecules such as DNA, proteins or membrane lipids is an important process during early carcinogenesis (Borrelli et al., 2007; Valko et al., 2006). Antioxidant activities of phytochemicals that counteract ROS or free radicals are therefore highly desirable for potential anti-cancer compounds. Some OSCs derived from *Allium* have been reported to possess an antioxidant activity (Borek, 2001). Due to their sulfur atoms, which can appear in up to ten different oxidation states (-2 to +6), these compounds can undergo redox-reactions (Cerella et al., 2009). Nishimura et al. reported that *Allium*-derived organic sulfides and sulfoxides form *in vivo* sulfoxides and sulfones, respectively, by

scavenging free hydroxyl (•OH) or •OOH radicals (Nishimura et al., 2000). Rabinkov and colleagues clearly demonstrated the radical scavenging ability of alliin and allicin by using the *in vitro* Fenton oxygen-radical generating system, in which the amount of •OH radicals, measured with the spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO), was significantly reduced in response to both OSCs (Rabinkov et al., 1998). Alliin was described as stronger superoxide scavenger than allylcysteine or allyldisulfide (Okada et al., 2005). By direct ESR detection Okada et al. provided evidence that allicin is a potent scavenger of peroxyl radicals (Okada et al., 2005). Structure-activity studies of the same authors revealed that the –S(O)S- group in combination with an allyl group is particularly important for the antioxidant activity of thiosulfinates (Okada et al., 2005). Several studies performed on bovine pulmonary artery endothelial cells revealed that AGE and SAC inhibited $O2^{\bullet-}$ and H_2O_2 formation as well as H_2O_2-induced lipid peroxidation (Yamasaki & Lau, 1997) (Wei Z., 1998). Particularly for the stable water-soluble sulfur species SAC and SAMC remarkable antioxidant properties are well documented. Thus, Imai et al. who investigated the antioxidant effects of AGE, a formulation especially enriched in both SAC and SAMC, provided evidence that these OSCs possess extraordinary radical scavenging activity (Imai et al., 1994). Counteracting ROS accumulation could also explain the beneficial effects of garlic in diseases different from cancer, in which ROS play a major role, for example cardiovascular and inflammatory diseases. In this respect, aqueous garlic extract, AGE and SAC are effective in the prevention of copper-induced LDL oxidation, a process implicated in the pathogenesis of atherosclerosis (Amagase et al., 2001; Galeone et al., 2006). AGE also protects rat liver microsomes from thiobarbituric acid-reactive substances (TBARS) formation (Horie et al., 1989). Other garlic-derived compounds, including lipophilic polysulfides and dithiins have also shown to act as antioxidants (Amagase et al., 2001), e.g. by preventing LOOH formation on human LDL (Nishimura et al., 2004). Nishimura et al. identified 3,4-dihydro-3-vinyl-1,2-dithiin as the compound with the most efficient antioxidant activity on human LDL at physiological concentration (Nishimura et al., 2004). Another strategy used by OSCs is the increase in the cellular antioxidant defense by upregulating/activating phase II detoxification enzymes (see Section 5.1.1) as well as alteration of the GSH redox cycle have been demonstrated in response to AGE and SAC (Geng, 1997). Oxidative stress is also linked to inflammation, while the latter is associated with carcinogenesis (Bartsch & Nair, 2006; Borrelli et al., 2007; Kundu & Surh, 2008). ROS are known to activate the NF-κB signalling pathway thereby causing aberrant expression of target genes, including pro-inflammatory cytokines and chemokines that are involved in tumorigenesis (Balkwill & Mantovani, 2001; Karin & Greten, 2005). Blocking of this pathway therefore plays a role in the antioxidant defense. Indeed, SAC was efficient in preventing NF-κB activation induced by both, tumor necrosis factor alpha and H_2O_2 in human T lymphocytes (Geng, 1997). DADS acts on the NF-κB pathway by blocking activation and nuclear translocation of p65 and p50 (Pratheeshkumar et al., 2010).

On the other hand, there are some publications reporting a pro-oxidant activity of diallylsulfides and ajoene. These studies suggested that polysulfide/ajoene-induced apoptotic cell death is caused by ROS accumulation and peroxide production (Aquilano et al., 2010; Charlier et al., 2010; Dirsch et al., 1998; Wang, H.C. et al., 2010a). DADS, DATS and DATTS and to a lesser extent the corresponding propylsulfides have been identified as hemolytic agents, as they induce oxidative damage in erythrocytes by the formation of H_2O_2 in the presence of GSH and haemoglobin (Munday et al., 2003).

5.3.2 Interaction with metalloproteins

Metalloproteins are indispensable for cells to achieve cancer prevention. Matrix metalloproteinases for example counteract carcinogenic processes like metastasis or angiogenesis by ensuring the consistency of the extracellular matrix, but may facilitate tumor invasion if overexpressed (Brew et al., 2000; Gomez et al., 1997; Luttun et al., 2000). Members of other metalloprotein classes including the cytochrome P450 family or proteins are involved in iron or drug/xenobiotic metabolism and activation of carcinogens (Sheweita, 2000; Shimada, 2006). The ability of garlic-derived OSCs to form complexes with metal ions might result in the inactivation of metallo-enzymes and therefore represents an important anti-cancer strategy of OSCs. Thiolates for example are exceptional ligands for various metal ions. Even if an experimental proof for direct binding of OSCs to metal ions is still missing, polysulfides are able to coordinate with several sulfur atoms at once and thus are likely to form metal complexes (Munchberg et al., 2007). In this respect, different OSCs are known to alter the activity of members of the cytochrome P450 family (see Section 5.1.1).

A family of zinc-dependent endopeptidases, named matrix metalloproteinases (MMPs) according to their primary function (Kessenbrock et al., 2010; Nagase & Woessner, 1999), is also affected by some garlic-derived OSCs (Ho et al., 2010; Meyer et al., 2004; Peng et al., 2010; Polette & Birembaut, 1998). Often, high expression levels of MMPs are detected in cancer cells, which further increase during tumor progression (Chambers & Matrisian, 1997). Such an imbalance, particularly of MMP9 (Egeblad & Werb, 2002) and MMP2 activity, is associated with cancer cell invasion and metastasis (Ahmed & Mohammed, 2011; Ellerbroek & Stack, 1999; Peng et al., 2010; Polette & Birembaut, 1998). MMPs therefore represent a crucial target in anti-cancer treatment. Meyer et al. found that DADS decreased MMP2 activity in a dose-dependent manner in HUVEC cells (Meyer et al., 2004). AM and SAC did not show any effect. DADS affected in addition MMP9 activity, whereas MMP1 and MMP3 activity/secretion were not altered by any of the OSCs. These results are in line with a study of Shin et al. who found DADS suppressing MMP2 and MMP9 in a time-dependent manner in prostate LNCaP cancer cells (Ho et al., 2010). As already mentioned above it has been speculated that perthiols arising from polysulfides might contribute to the biological activity of the latter. Similar to thiols, RSxH species serve as good ligands for zinc, copper, iron or other metal ions (Munchberg et al., 2007). Based on their low pK_a values, RSxH species are expected to strongly bind free or protein-bound metal ions (Munchberg et al., 2007). Instead of binding directly to the active site metal ion (Schneider, T. et al., 2011a), they might also form low molecular weight complexes in the cytosol. Due to these disturbances of metal homeostasis the pool of free (i.e. unbound) metal ions is diminished and impairs *de novo* synthesis of functioning metalloproteins (Munchberg et al., 2007). As free adventitious transition metal ions are also involved in oxidative stress (Bush, 2000), the OSC-induced reduction of the cytosolic metal ion pool contributes to the antioxidant effect of these sulfur compounds.

5.4 Modulation of histone acetylation

Recent studies have suggested the modulation of histone acetylation as one of the mechanisms involved in the anti-cancer activity of garlic (Druesne-Pecollo & Latino-Martel, 2011). In eukaryotic cells, histones are proteins responsible for the DNA condensation and thus play an important role in the regulation of gene expression. Histones are subjected to

post-translational modifications, including acetylation, which is a reversible process occurring on lysine residues in the N-terminal domain of the protein. In cancer cells, various genes are abnormally expressed due to epigenetic modifications of the chromatin, leading to the tumor phenotype. Controlling chromatin remodeling and gene expression is a relevant strategy for anti-cancer drug development (Espino et al., 2005). Few studies have described the ability of OSCs to increase histone acetylation. Lea et al. reported for the first time the increased acetylation of histones H3 and H4 in mouse erythroleukemia DS19 and human leukemia K562 after treatment with DADS and AM (Lea et al., 1999). Then, the same group has confirmed those effects *in vivo* as histone acetylation was induced in liver and Morris hepatoma 7777 in rats treated with DADS and AM (Lea & Randolph, 2001). Data suggested a direct link between histone hyperacetylation and the antiproliferative activity of some garlic components. First, increased histone acetylation was obtained for concentrations similar to those used to inhibit cell proliferation of DS19, human colon cancer Caco-2 cells and human breast cancer T47D cells (μM range for allicin and SAMC, mM range for allylphenylsulfone and SAC) (Lea et al., 2002). In this study, the authors failed to characterize the mechanism involved as no inhibitory effect was observed on histone deacetylase (HDAC) activity, enzymes which repress the histone acetylation. Conversely, Druesne et al. confirmed the ability of DADS and AM to inhibit HDAC activity in a cell-free assay (29 % and 92 % of HDAC inhibition in presence of 200 mM of each compound, respectively) (Druesne et al., 2004). Recently, *in vitro* assay and molecular modeling have led to the identification of AM as the most potent competitive inhibitor of HDAC (Ki=24 □M for HDAC8). HDAC inhibition appeared to be responsible for the hyperacetylation of the p21 gene promoter (Nian et al., 2009; Nian et al., 2008).

Another important consideration is the chemopreventive activity of chromatin remodeling molecules. Indeed, it has been shown *in vivo* that DADS administration to rat effectively induced acetylation of histones H3 and H4 in non-tumor colonocytes. Results from microarray analysis indicated that DADS was able to modulate the expression of 49 genes (24 h after the end of DADS perfusion), mostly implicated in cell cycle regulation, DNA repair, cellular adhesion (Druesne-Pecollo et al., 2007). These results suggest a potential link between the modulation of gene expression, through histone hyperacetylation, and the chemopreventive activity of OSCs. Further investigations are needed to determine whether garlic components could be effective in histone acetylation, and thus in cancer prevention, at doses achievable in human diet.

5.5 Induction of apoptosis

5.5.1 Activation of the mitochondrial apoptotic pathway

A number of studies document the ability of OSCs to induce apoptosis. Apoptosis is an active form of cell death by which cells organize their self-elimination. It may be part of a programmed physiological event, including tissue remodeling and turn over; besides, irreversibly damaged cells following stress activate this mechanism. The apoptotic program is accomplished by the triggering different cascades of biochemical events. Two main intracellular routes take place (Coppola & Ghibelli, 2000). The intrinsic or mitochondrial pathway typically starts as a consequence of cellular damage. It requires the crucial involvement of pro- and anti-apoptotic members of the B-cell lymphoma (Bcl-2) family, which control the permeabilization of the outer mitochondrial membrane. The resulting

release of cytochrome c (and other additional factors) into the cytoplasm promotes caspase cleavage/activation, whose most upstream implicated member is caspase-9. The extrinsic or physiological pathway is mediated by the stimulation of specific death receptors on the plasma membrane. Once stimulated by their ligands, they unleash an ordered intracellular multi-step signaling, which includes the formation of the cell death domain and the activation/cleavage of caspase-8. The two pathways are interconnected.

The activation of the extrinsic or the intrinsic apoptotic pathways is the target of most chemotherapeutic agents. OSCs derived from *Allium* species induce apoptosis by activating the intrinsic apoptotic pathway. The release of cytochrome c from mitochondria, the decreased level of the anti-apoptotic protein Bcl-2 and the activation of caspase 9/3 are common hallmarks (Cerella et al., 2011; Scherer et al., 2009). The findings concern very heterogeneous cancer cell models, ranging from adherent (e.g., prostate, lung, colon, breast, cervical and thyroid) to non-adherent (e.g., various leukemia and lymphomas) cancer cell lines.

A large body of evidence shows a strong correlation between the number of sulfur atoms and the apoptogenic potential manifested by OSCs (see Section 3.1). Less defined, instead, is the contribution of the length of the sulfur chain in the anti-cancer role played by OCSs. Studies comparing the effects of diallylpolysulfides deriving from garlic, which bring an unsatured (alkenyl) chain, with propylpolysulfides extracted from onion, which contain a saturated alkyl group, show marginal differences in their apoptogenic potential: generally, molecules containing the same number of sulfur atoms, show also a similar impact on different cancer cell models (Cerella et al., 2011). In contrast, Hosono and colleagues have provided evidence by using a colon cancer cellular model HT29 that the alkenyl group impacts intracellular targets (see also section below) much stronger than the corresponding compound with an alkyl group (Hosono et al., 2008). This result may lead in this cell system to a potential differential role also on apoptosis, which, however, was not yet investigated. Further studies are required to explain cell-type specific responses to OSC.

5.5.2 Mechanisms implicated in G2/M arrest

In all instances, apoptosis is preceded by alterations in the progression of the cell cycle, consisting in a G2/M arrest (section 5.2). The activation/up-regulation of the tumor suppressor p53 has been mentioned since very early studies on apoptosis as a crucial and very upstream event (Shen & White, 2001). The arrest in G2/M induced by OSCs may be compatible with an involvement of p53 to activate apoptosis. p53 expression has been effectively found upregulated in studies performed with garlic extracts (Hong et al., 2000) (De Martino et al., 2006), as well as in a number of investigations based on the use of purified sulfur compounds: DAS (Hong et al., 2000), DADS (Bottone et al., 2002; Hong et al., 2000; Pratheeshkumar et al., 2010; Song et al., 2009), DATS (Malki et al., 2009) and SAMC (Lee, 2008). p53 upregulation, however, seems to be a dispensable event in the apoptogenic activity of OSCs, since the use of p53-mutated or deleted cancer cell models has clearly demonstrated that OSC-induced apoptosis similarly takes independently of wild-type p53 (Busch et al., 2010; Lee, 2008; Xiao et al., 2009b).

A more careful observation of the cellular morphology after OSCs treatment suggests an arrest of the cell cycle at early steps of mitosis and more precisely during the pro-metaphase.

Accordingly, the nuclear chromatin partially condenses and acquires a dotted pattern. The pattern of modulations that OCSs produce on cell cycle-related proteins confirms these conclusions. The accumulation of cyclin B1 correlates with the down-regulation of CDC25B and 25C, which control the phosphorylation status and the activation of cyclin-dependent kinase (Cdk1) (see section 5.2). Besides, treated cells present a high positivity to the phosphorylated form of histone H3, at the level of ser 10 (H3P) (Cerella et al., 2009; Herman-Antosiewicz & Singh, 2005). The degradation of cyclin B1 and H3P is an indispensable event to progress during mitosis into the anaphase. Therefore, their accumulation indicates that OSCs prevent the transition to this step and, by this way, may trigger the mitochondrial apoptotic pathway.

5.5.3 Alterations induced by OSCs on the MT network as a causative event of apoptosis

Arrest at early steps of mitosis may be the consequence of aberrations occurring during the DNA synthesis; alternatively, it occurs after alteration of the MT network. A few reports analyzed the potential of OSCs to cause DNA damage (Wang, H.C. et al., 2010a). In most instances, this is not an early event, but rather appearing tightly linked to the onset of apoptosis. Consequently, DNA damage is not generally considered as the causative event determining the OSC-induced apoptosis. A large body of evidence, conversely, indicates that OSCs induce strong alterations of MT re-arrangement (see figure 3 and section 3.2). This property is common to different natural occurring OSCs manifesting apoptogenic properties on the different cancer cell models (Aquilano et al., 2010; Hosono et al., 2005; Li et al., 2002a; Prager-Khoutorsky et al., 2007; Xiao et al., 2005; Xiao et al., 2003).

Despite the common observation that the MT network is affected by OSCs, there is no general consensus about the molecular events leading to these alterations. Canonically, stress-induced apoptosis has been always associated with early alterations in the cellular redox state (Coppola & Ghibelli, 2000). Changes in the intracellular GSH content and the generation of ROS are commonly assumed to act as crucial events in determining the commitment to intrinsic apoptosis. Previously published data already showed that a pretreatment with antioxidants, mainly with NAC, counteracts OSCs-induced apoptosis (Dirsch et al., 1998). Moreover, NAC prevents the G2/M arrest (Wu, X.J. et al., 2005). Similar results have provided the strongest evidence that the induction of apoptosis by OSCs effectively depends on the alterations of the cell cycle. Besides, the generation of ROS has been documented in some studies by the use of the fluorescent tool 2,7-dichlorodihydrofluorescein diacetate (H_2DCFDA) upon incubation of the cells with DADS and DATS (Filomeni et al., 2003; Kim, Y.A. et al., 2007; Sriram et al., 2008). Consequently, ROS have been considered by a number of studies as the determining event for cell cycle arrest and apoptosis induced by OSCs. As previously discussed, agents such as NAC may counteract OSCs-dependent cell cycle alterations and, therefore apoptosis, simply by acting as donors of thiols and thus competing with the intracellular thiols for the interaction with OSCs. Several pieces of evidence support this model. First, the direct ability of NAC to prevent the OSCs-induced tubulin depolymerization (see section 3.2). Second, ROS production has not always been detected, even if apoptosis was induced (De Martino et al., 2006). The debate, however, remains open for further discussion. The over-expression of the

enzyme SOD has been shown in the neuroblastoma SH-SY5Y cells to counteract apoptosis (Filomeni et al., 2003). Besides, pre-incubation of cells with catalase, which buffers H_2O_2 production, counteracted apoptosis induced by DADS in the HL-60 leukemia cell line (Kwon et al., 2002) and in T24 human bladder cancer cells (Lu et al., 2004). Similarly, over-expression of catalase in the prostate cancer cell line DU145 protects against apoptosis induced by DATS (Xiao et al., 2004). A conceivable interpretation is that ROS species may be generated downstream of the formation of intracellular thiols after OSC treatment and play a determinant role depending on the cell-specific antioxidant reservoir.

5.5.4 Modulation of Bcl-2 family members by OSCs

OSCs modulate several proteins belonging to the Bcl-2 family. The anti-apoptotic Bcl-2 protein has been described to be downregulated. This, in turn, increases Bcl-2-associated X protein (Bax)/Bcl-2 ratio, thus promoting apoptosis (Karmakar et al., 2007). The downregulation of Bcl-2 is more precisely the consequence of a protein cleavage. In our laboratory, we have shown that DATTS promotes Bcl-2 proteolysis (Cerella et al., 2009). This finding is in line with previous results collected with Z-ajoene (Li et al., 2002b) and DATS (Xiao et al., 2004). Bcl-2 cleavage is preceded by phosphorylation. Xiao and colleagues published that Bcl-2 phosphorylation occurs after treatment with DATS in human prostate cancer cells PC-3 and DU145 (Xiao et al., 2004). Similarly, the expression of the anti-apoptotic Bcl-xL protein is modulated by DADS in breast cancer cells (Nakagawa et al., 2001); in line with these results, we have described that the downregulation of B-cell lymphoma-extra large (Bcl-xL) protein upon treatment with DATTS in human leukemia cells (Cerella et al., 2009). Specific Bcl-2 homology domain 3 (BH3)-only proteins seem to be modulated by OSCs by post-translational modification or by intracellular redistribution. Unphosphorylated Bcl-2-associated death promoter (Bad) sequesters Bcl-xL and Bcl-2 thus favoring Bax and Bcl-2 homologous antagonist killer (Bak) activation (Yang et al., 1995). Dephosphorylation of Bad has been documented after DATTS treatment (Cerella et al., 2009). Besides, the translocation of Bad to the mitochondria occurs in prostate cancer cells upon treatment with DATS (Xiao & Singh, 2006). When breast cancer MDA-MB-435 cells were exposed to water-soluble extracts (Lund et al., 2005), expression level, phosphorylation and mitochondrial localization of different isoforms of the BH3-only protein BimEL were altered. Interestingly, BimEL in normal physiological conditions is anchored to (and sequestered by) the MT complex (Chen, D. & Zhou, 2004; Lund et al., 2005). This may provide interesting insights about how the MT network is disrupted and the subsequent activation of the mitochondrial pathway may be interconnected.

The permeabilization of the outer mitochondrial membrane is an event controlled by the two pro-apoptotic family members, Bax and Bak, which orchestrate the formation of specific channels (Antignani & Youle, 2006). OSCs activate Bax and Bak (Cerella et al., 2009; Kim, Y.A. et al., 2007; Xiao et al., 2006a; Xiao et al., 2009a). Interestingly, time-course analyses have evidenced that Bak might be activated before Bax (Cerella et al., 2009). The activation of Bax fits the activation of caspases and the appearance of massive apoptosis (Cerella et al., 2009), thus suggesting that its activation is crucial for the completion of the apoptotic program. In other instances, modifications in the levels of Bak and Bax have been detected, with Bak being affected much earlier than Bax (Kim, Y.A. et al., 2007). This evidence is in favor of non-redundant roles of Bax and Bak during OSCs-induced apoptosis. How Bax and

Bak may promote the formation of channels and the mitochondrial outer membrane permeabilization is still unknown. Similarly, differential roles played by Bax and Bak during apoptosis remain to be determined. Intriguingly, the chemotherapeutic agent vinblastine, which affects the MT network and induces mitotic arrest, also modulates Bak at early timepoints (Upreti et al., 2008). These results may suggest a specific interplay between Bak and Bax typically activated by tubulin-affecting agents. OSCs might conceivably represent an interesting tool to investigate and to clarify some aspects that belong to essential issues of apoptosis research.

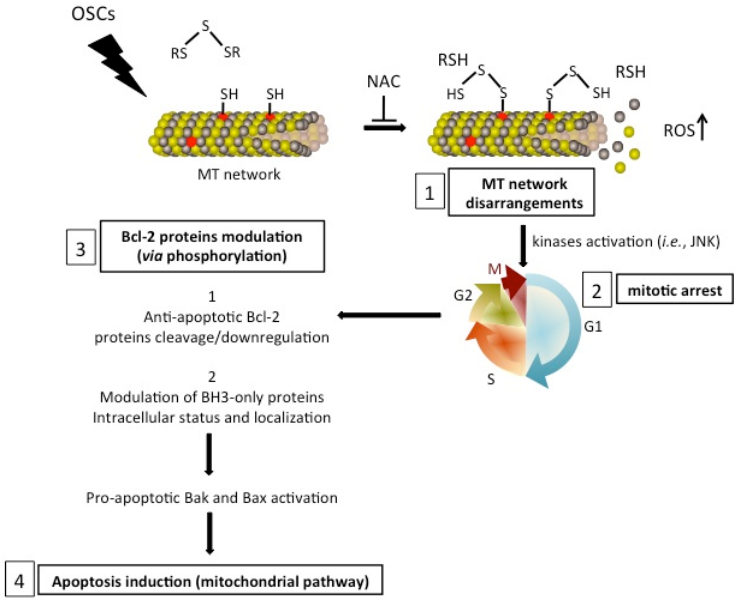

Fig. 3. Mechanisms involved in the apoptogenic properties of OSCs
The induction of apoptosis by OSCs occurs *via* a well-ordered set of events. The first target is the MT network whose modification leads to G2/M arrest of the cells in early mitosis. Kinases act as sensors of the stress occurring to MT. Once activated, they translate the stress signal to the Bcl-2 family proteins, by modulating anti-apoptotic and BH3-only proteins *via* phosphorylation. This impact on Bcl-2 modulatory proteins unleashes the pro-apoptotic Bak and Bax, which are now free to trigger the mitochondrial apoptotic pathway (modified from (Cerella et al., 2009).

5.5.5 Involvement of kinases in OSCs-mediated apoptosis

Different Bcl-2 family members are modulated by OSCs *via* protein phosphorylation. In addition, different kinases are activated upon treatment with several OSCs. The activation of c-Jun N-terminal kinase (JNK) appears as the most relevant for the apoptogenic potential of OSCs. JNK activation has been commonly reported upon DADS treatment in neuroblastoma SH-SY5Y (Filomeni et al., 2003) and breast cancer MCF-7 cells (Lei et al., 2008); in prostate PC-3 and DU145 cells in response to DATS (Xiao et al., 2004); by SAMC in colon cancer SW480 cells (Xiao et al., 2003). JNK activation is required for the apoptogenic activity of

OSCs, since the specific JNK inhibitor SP600125 is able to exert a protective effect (Wu, X.J. et al., 2009; Xiao et al., 2003). JNK activation and Bcl-2 phosphorylation are events that accompany changes in the MT dynamics (Mollinedo & Gajate, 2003). This suggests an intermediate role of JNK as a transducer of the stress produced by OSCs between the MT network and Bcl-2 (and possibly to other Bcl-2 family members). Alternatively, JNK activation has been explained as a consequence of ROS production, which in turn, may impair the ability of the JNK guardian GST to sequester and prevent the activation of the kinase (Filomeni et al., 2003). Further MAP kinases are activated by OSCs. Phosphorylation of extracellular signal-regulated kinase (ERK) has been frequently reported (Xiao et al., 2004). Similarly, there is evidence of the activation of p38 mitogen-activated protein kinase (MAPK) (Das et al., 2007; Zhang, Y.W. et al., 2006) and Akt kinases (Wang, Y.B. et al., 2010b). The results, however, are not conclusive because their inhibition did not give univocal results. Figure 3 reports a model of interactions between kinases and the other factors/ events modulated during OSCs treatment.

5.6 Inhibition of metastatic process and differentiation

Metastasis is a major cause of death in cancer patients. Tumor invasion is a multistep process involving the detachment of cancer cells at the site of the primary tumor, the entry into the systemic circulation and the invasion of new tumor sites. These different steps require multiple molecular events including loss of adhesion and motility of cancer cells, and stimulating angiogenesis (which appears to be essential for cancer cell spread). First, several *in vitro* studies have reported that garlic extracts and some of its components are able to affect cancer cell motility and invasiveness. In 2002, Hu and coworkers published a first report showing that AGE suppressed rat sarcoma cell migration *in vitro* (Hu et al., 2002). Referring to these results, the antimetastatic activity of purified OSCs (e.g., DADS, SAC) was investigated. Shin and colleagues demonstrated that DADS reduced the motility of the prostate LNCaP cancer cell model by increasing the tightness of the tight junctions and by reducing the activity, the mRNA and protein levels of MMP2 and 9 (Shin et al., 2010). SAC also altered breast tumor MDA-MB-231 cell adhesion and invasion through the induction of adhesion protein E-cadherin expression and the decrease of MMP2 activity (Gapter et al., 2008). Recently, Lai et al. have reported that the inhibition of MMP2, 7 and 9 in colo 2005 cells by DAS, DADS and DATS were mediated through the downregulation of phosphoinositide 3-kinase (PI3K), Rat sarcoma (RAS), MAP kinase kinase kinase (MEKK) 3, MAP kinase kinase (MKK7), ERK1/2, JNK1/2 and p38 MAPK (Lai et al., 2011). Moreover, suppression of angiogenesis, a process leading to the formation of new blood vessels from the pre-existing vascular network, has also been reported. This process is essential in cancer development as it takes part in primary tumor growth (*i.e.*, provision of oxygen and nutrients) and in spreading to distant sites, leading to metastasis. Tumor cells are known to promote angiogenesis through the production of several growth factors, which stimulate endothelial cell proliferation and migration (for a recent review, see (Makrilia et al., 2009)). In 2006, studies have shown that AGE and DATS inhibited the proliferation and the migration of endothelial cells as well as the capillary-like tube formation *in vitro* (Matsuura et al., 2006; Xiao et al., 2006b). For example, Xiao et al. reported that DATS exerted its antiangiogenic activity through the inhibition of vascular endothelial growth factor (VEGF) secretion, the reduction of VEGF receptor 2 expression and the inactivation of Akt signaling pathway in human umbilical vein endothelial cells (HUVEC) (Xiao et al., 2006b).

In vivo studies have confirmed the evidence supporting the antimetastatic properties of garlic *in vitro*. Oral administration of SAMC to mice bearing prostate tumors reduced the number of pulmonary metastasis by 85% and completely abolished their presence in adrenal cells (Howard et al., 2007). In another study, Singh and coworkers have shown that oral administration of DATS to TRAMP mice decreased the development of pulmonary metastasis (about 50% compared with the control mice) without inhibiting angiogenic features at the tested dose (Singh et al., 2008). Despite the different evidence obtained *in vitro*, the molecular events implicated in the antimetastatic activity of OSCs remain to be clarified.

Cancer cells are characterized by the loss of growth control mechanisms and thus remain in a less differentiated, immature state. One important therapy approach is therefore the induction of differentiation in order to restrain the cancer cell proliferation. In this respect, garlic oil was described to induce differentiation in human gastric cancer BGC-823 (Brew et al., 2000) and HL-60 promyelocytic leukemia cells (Seki et al., 2000). The ability to induce cancer cell differentiation could be linked to DADS and SAC. Studies on DADS found that upregulation of p21(WAF) and acetylated histones H3 and H4 is associated with the induction of differentiation in HL-60 cells (Ling et al., 2006) while in MGC803 gastric cancer cells alterations of the ERK1/2 signaling pathway are involved in this process (Chu et al., 2006). Abnormal expression of cytokeratins (marker of epithelial cell differentiation), which is connected to malignant progression, was restored by SAC administration in hamster buccal pouch carcinogenesis in Syrian hamsters (Balasenthil et al., 2003). Other studies reported that SAC upregulated E-cadherin expression in MDA-MB-231 breast tumor and PCa prostate cancer cells (Chiang et al., 2006; Gapter et al., 2008). On the other hand, in former studies, no differentiation markers could be detected in neuroblastoma or melanoma cells upon SAC treatment (Takeyama et al., 1993; Welch et al., 1992).

6. Bioavailability and pharmacokinetics of OSCs

Little is known from preclinical and clinical studies about the bioavailability, the metabolism and the excretion of garlic ingredients after consumption. Guo and coworkers have reported that pure alliin is absorbed *in vivo* as it can be detected in stomach (7.2%), intestine (22.4%) and liver (2.5%) after oral administration to mice. In this study, neither allicin nor other degradation products, such as DAS, DADS, vinyldithiins, were found. This clearly indicates that alliin itself is never metabolized, in absence of alliinase, even by liver enzymes (Guo et al., 1990). *Ex vivo* experiments, on isolated perfused rat liver, have suggested that allicin is not a biologically active component of garlic. After infusion at low concentrations, allicin bound to liver epithelium, reached the hepatic first-pass and was rapidly metabolized into DADS and AM, both compounds detectable in hepatic tissue and bile; allicin instead was not found (Egen-Schwind et al., 1992b). Another study of the same authors confirmed that the metabolism of allicin occurred in liver as it was rapidly transformed in presence of liver homogenate (Egen-Schwind et al., 1992a). Experiments using intraperitoneal injection of [^{35}S]-labeled DADS in mice revealed that a maximum of radioactivity (70% of the total amount) was detected in the liver 90 min after administration. Actually, mostly [^{35}S]-labeled sulfate (80% of the radioactivity of the liver) was detected suggesting that the metabolism of DADS occurs in this organ (Pushpendran et al., 1980). Germain and coworkers have reported that DADS was mainly transformed into AM, AMS,

allylmethylsulfoxide and allylmethylsulfone when orally administrated to rats (Germain et al., 2002). Pharmacokinectics of vinyldithiins have also been studied in animal models. Both 2-vinyl-4H-1,3-dithiin and 3-vinyl-4H-1,2-dithiin were found in serum, kidney and fat tissue after oral administration to rats, whereas only 2-vinyl-4H-1,3-dithiin was found in liver. The latter was more rapidly eliminated probably due to its lower lipophilicity (Egen-Schwind et al., 1992a). Bioavailability of SAC, a main constituent of AGE, was more extensively studied in animal and human. SAC achieved the intestinal first-pass and occurred in plasma, liver and kidney. Its bioavailability was close to 100% when administrated to rat, mouse and dog. Interestingly, concentrations detected in blood correlated well with the doses of oral intake, suggesting SAC as a biological active component of garlic. Then, SAC was excreted in urine as N-acetylated metabolites, indicating a metabolizing-process mediated through N-acetyl transferases (Nagae et al., 1994). Comparable results have been obtained from clinical studies (Jandke & Spiteller, 1987; Rosen et al., 2001).

7. Ongoing clinical trials

At the moment, few clinical trials are ongoing (information available on www.clinicaltrials.gov). These studies aim at elucidating the bioavailability of garlic in humans and evaluating its benefits in chemoprevention and as supplement in cancer treatment. A study with healthy patients deals with the bioavailability of allicin from garlic supplements (powder) and garlic clove (raw, cooked, processed). Concerning the chemopreventive benefit of garlic consumption, some investigators are recruiting healthy volunteers to evaluate the way by which garlic reduces cancer risk (analysis of a panel of cancer biomarkers in control *vs.* treated groups). Finally, two studies are conducted to assess the potential benefits of co-administration of garlic and conventional chemotherapeutics in cancer patients. As example, we can cite a clinical trial studying how garlic intake affects docetaxel treatment in locally advanced or metastatic breast cancer patients. Another phase II study, gathering 45 patients with aggressive follicular lymphoma (stage III/IV), has been started to assess the ability of garlic extract to impact apoptosis and cancer cell proliferation.

8. Conclusions

A large body of evidence supports the anti-cancer potential of OSCs from *Allium* species. The huge amount of data is consistent with a future dual potential clinical application of selected compounds, in chemoprevention as well as in chemotherapy. The sulfur component is mostly responsible of their chemical properties. The fact that many enzymes and proteins are regulated by modifications occurring at the level of redox-sensitive cysteine residues accounts for their versatility and multitasking potential. So far a good part of the path in the elucidation of the mechanisms responsible for OSCs anti-cancer activities has been covered.

Some important challenges need still to be faced. First, the impact of OSCs on cell cycle raises the question if purified agents from *Allium* species may be nevertheless toxic for not-malignant proliferating cells, at the concentrations tested *in vitro*. In this sense, indepth investigations confirming the differential effects of OSCs on cancer *vs.* healthy cells are absolutely required. Second, the fact that cancer of the digestive tract and prostate appear as the most sensitive ones to the chemopreventive effects of *Allium* vegetables intake may

reflect a problem of their stability. Tissues/organs, which are in inner connection with the digestive/urinary systems, are obviously exposed to higher concentrations of biologically active products deriving from the degradation/catabolism of any ingested food or supplements. This encourages any efforts aimed at increasing the stability of these compounds, hopefully without increasing also their toxicity. Finally, the conjugation of molecules specifically recognizing and binding to cancer cells together with the enzyme alliinase could generate high concentrations of alliin right at the tumor site and may represent an interesting attempt to overcome both limiting factors (Appel et al., 2011).

9. Acknowledgements

C.C., M.K. and E.V. are recipients of postdoctoral Télévie, ''Recherches Scientifiques Luxembourg'' (RSL; UNBS1450 grant) and FNR grants, respectively. Research at the Laboratoire de Biologie Moléculaire et Cellulaire du Cancer (LBMCC) is financially supported by the ''Recherche Cancer et Sang'' foundation, by the RSL association, by ''Een Haerz fir kriibskrank Kanner'' association, by the Action Lions ''Vaincre le Cancer''association and by Télévie Luxembourg. Research in CJ's lab was supported financially by the University of Saarland and the Ministry of Economics and Science of Saarland. The research in both labs has received funding from the European Community's 7th Framework Program under grant agreement number 215009 - RedCat and the Interreg IVA program (35 GR 1 1051) Corena-Network of the European Union. Editing and print costs are covered by the Fonds National de la Recherche (FNR), Luxembourg.

10. References

Agarwal, K.C. (1996). Therapeutic actions of garlic constituents. *Medicinal research reviews*, 16, 1, (Jan 1996), pp. (111-124), ISSN 0198-6325

Ahmed, M.M. & Mohammed, S.H. (2011). Matrix metalloproteinases 2 and 9 in situ mRNA expression in colorectal tumors from Iraqi patients. *Indian journal of pathology & microbiology*, 54, 1, (Jan-Mar 2011), pp. (7-14), ISSN 0377-4929

Alkreathy, H.; Damanhouri, Z.A.; Ahmed, N.; Slevin, M.; Ali, S.S. & Osman, A.M. (2010). Aged garlic extract protects against doxorubicin-induced cardiotoxicity in rats. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, 48, 3, (Mar 2010), pp. (951-956), ISSN 0278-6915

Amagase, H.; Petesch, B.L.; Matsuura, H.; Kasuga, S. & Itakura, Y. (2001). Intake of garlic and its bioactive components. *The Journal of nutrition*, 131, 3s, (Mar 2001), pp. (955S-962S), ISSN 0022-3166

Amagase, H. (2006). Clarifying the real bioactive constituents of garlic. *The Journal of nutrition*, 136, 3 Suppl, (Mar 2006), pp. (716S-725S), ISSN 0022-3166

Andorfer, J.H.; Tchaikovskaya, T. & Listowsky, I. (2004). Selective expression of glutathione S-transferase genes in the murine gastrointestinal tract in response to dietary organosulfur compounds. *Carcinogenesis*, 25, 3, (Mar 2004), pp. (359-367), ISSN 0143-3334

Antignani, A. & Youle, R.J. (2006). How do Bax and Bak lead to permeabilization of the outer mitochondrial membrane? *Current opinion in cell biology*, 18, 6, (Dec 2006), pp. (685-689), ISSN 0955-0674

Anwar, A.; Burkholz, T.; Scherer, C.; Abbas, M.; Lehr, C.M.; M., D. & Jacob, C. (2008). Naturally occurring reactive sulfur species, their activity against Caco-2 cells, and possible modes of biochemical action *Journal of Sulfur Chemistry*, 29, 3-4, (June-August 2008), pp. (251-268), ISSN 1741-5993

Appel, E.; Rabinkov, A.; Neeman, M.; Kohen, F. & Mirelman, D. (2011). Conjugates of daidzein-alliinase as a targeted pro-drug enzyme system against ovarian carcinoma. *Journal of drug targeting*, 19, 5, (Jun 2011), pp. (326-335), ISSN 1026-7158

Aquilano, K.; Vigilanza, P.; Filomeni, G.; Rotilio, G. & Ciriolo, M.R. (2010). Tau dephosphorylation and microfilaments disruption are upstream events of the anti-proliferative effects of DADS in SH-SY5Y cells. *Journal of cellular and molecular medicine*, 14, 3, (Mar 2010), pp. (564-577), ISSN 1582-1838

Arora, A. & Shukla, Y. (2002). Induction of apoptosis by diallyl sulfide in DMBA-induced mouse skin tumors. *Nutrition and cancer*, 44, 1, 2002), pp. (89-94), ISSN 0163-5581

Arora, A.; Seth, K. & Shukla, Y. (2004). Reversal of P-glycoprotein-mediated multidrug resistance by diallyl sulfide in K562 leukemic cells and in mouse liver. *Carcinogenesis*, 25, 6, (Jun 2004), pp. (941-949), ISSN 0143-3334

Balasenthil, S.; Rao, K.S. & Nagini, S. (2002). Garlic induces apoptosis during 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis. *Oral oncology*, 38, 5, (Jul 2002), pp. (431-436), ISSN 1368-8375

Balasenthil, S.; Rao, K.S. & Nagini, S. (2003). Altered cytokeratin expression during chemoprevention of hamster buccal pouch carcinogenesis by S-allylcysteine. *Polish journal of pharmacology*, 55, 5, (Sep-Oct 2003), pp. (793-798), ISSN 1230-6002

Balkwill, F. & Mantovani, A. (2001). Inflammation and cancer: back to Virchow? *Lancet*, 357, 9255, (Feb 17 2001), pp. (539-545), ISSN 0140-6736

Ban, J.O.; Lee, H.S.; Jeong, H.S.; Song, S.; Hwang, B.Y.; Moon, D.C.; Yoon do, Y.; Han, S.B. & Hong, J.T. (2009). Thiacremonone augments chemotherapeutic agent-induced growth inhibition in human colon cancer cells through inactivation of nuclear factor-{kappa}B. *Molecular cancer research : MCR*, 7, 6, (Jun 2009), pp. (870-879), ISSN 1541-7786

Bartsch, H. & Nair, J. (2006). Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage, and repair. *Langenbeck's archives of surgery / Deutsche Gesellschaft fur Chirurgie*, 391, 5, (Sep 2006), pp. (499-510), ISSN 1435-2443

Bhuvaneswari, V.; Rao, K.S. & Nagini, S. (2004). Altered expression of anti and proapoptotic proteins during chemoprevention of hamster buccal pouch carcinogenesis by tomato and garlic combination. *Clinica chimica acta; international journal of clinical chemistry*, 350, 1-2, (Dec 2004), pp. (65-72), ISSN 0009-8981

Bianchini, F. & Vainio, H. (2001). Allium vegetables and organosulfur compounds: do they help prevent cancer? *Environmental health perspectives*, 109, 9, (Sep 2001), pp. (893-902), ISSN 0091-6765

Block, E. (1985). The chemistry of garlic and onions. *Scientific American*, 252, 3, (Mar 1985), pp. (114-119), ISSN 0036-8733

Block E., A.S., Jain M.K., Crecely R.W., Apitz-Castro R., Cruz M.R. (1984). (E,Z)-Ajoene: a potent antithrombotic agent from garlic. *Journal of the American Chemical Society*, 106, 1984), pp. (8295-8296), ISSN

Borek, C. (2001). Antioxidant health effects of aged garlic extract. *The Journal of nutrition*, 131, 3s, (Mar 2001), pp. (1010S-1015S), ISSN 0022-3166

Borrelli, F.; Capasso, R. & Izzo, A.A. (2007). Garlic (Allium sativum L.): adverse effects and drug interactions in humans. *Molecular nutrition & food research*, 51, 11, (Nov 2007), pp. (1386-1397), ISSN 1613-4125

Bose, C.; Guo, J.; Zimniak, L.; Srivastava, S.K.; Singh, S.P.; Zimniak, P. & Singh, S.V. (2002). Critical role of allyl groups and disulfide chain in induction of Pi class glutathione transferase in mouse tissues in vivo by diallyl disulfide, a naturally occurring chemopreventive agent in garlic. *Carcinogenesis*, 23, 10, (Oct 2002), pp. (1661-1665), ISSN 0143-3334

Bottone, F.G., Jr.; Baek, S.J.; Nixon, J.B. & Eling, T.E. (2002). Diallyl disulfide (DADS) induces the antitumorigenic NSAID-activated gene (NAG-1) by a p53-dependent mechanism in human colorectal HCT 116 cells. *The Journal of nutrition*, 132, 4, (Apr 2002), pp. (773-778), ISSN 0022-3166

Brady, J.F.; Li, D.C.; Ishizaki, H. & Yang, C.S. (1988). Effect of diallyl sulfide on rat liver microsomal nitrosamine metabolism and other monooxygenase activities. *Cancer research*, 48, 21, (Nov 1 1988), pp. (5937-5940), ISSN 0008-5472

Brady, J.F.; Ishizaki, H.; Fukuto, J.M.; Lin, M.C.; Fadel, A.; Gapac, J.M. & Yang, C.S. (1991). Inhibition of cytochrome P-450 2E1 by diallyl sulfide and its metabolites. *Chemical research in toxicology*, 4, 6, (Nov-Dec 1991), pp. (642-647), ISSN 0893-228X

Brew, K.; Dinakarpandian, D. & Nagase, H. (2000). Tissue inhibitors of metalloproteinases: evolution, structure and function. *Biochimica et biophysica acta*, 1477, 1-2, (Mar 7 2000), pp. (267-283), ISSN 0006-3002

Buiatti, E.; Palli, D.; Decarli, A.; Amadori, D.; Avellini, C.; Bianchi, S.; Biserni, R.; Cipriani, F.; Cocco, P.; Giacosa, A. & et al. (1989). A case-control study of gastric cancer and diet in Italy. *International journal of cancer. Journal international du cancer*, 44, 4, (Oct 15 1989), pp. (611-616), ISSN 0020-7136

Busch, C.; Jacob, C.; Anwar, A.; Burkholz, T.; Aicha Ba, L.; Cerella, C.; Diederich, M.; Brandt, W.; Wessjohann, L. & Montenarh, M. (2010). Diallylpolysulfides induce growth arrest and apoptosis. *International journal of oncology*, 36, 3, (Mar 2010), pp. (743-749), ISSN 1019-6439

Bush, A.I. (2000). Metals and neuroscience. *Current opinion in chemical biology*, 4, 2, (Apr 2000), pp. (184-191), ISSN 1367-5931

Cerella, C.; Scherer, C.; Cristofanon, S.; Henry, E.; Anwar, A.; Busch, C.; Montenarh, M.; Dicato, M.; Jacob, C. & Diederich, M. (2009). Cell cycle arrest in early mitosis and induction of caspase-dependent apoptosis in U937 cells by diallyltetrasulfide (Al2S4). *Apoptosis : an international journal on programmed cell death*, 14, 5, (May 2009), pp. (641-654), ISSN 1360-8185

Cerella, C.; Dicato, M.; Jacob, C. & Diederich, M. (2011). Chemical properties and mechanisms determining the anti-cancer action of garlic-derived organic sulfur compounds. *Anti-cancer agents in medicinal chemistry*, 11, 3, (Mar 1 2011), pp. (267-271), ISSN 1871-5206

Challier, B.; Perarnau, J.M. & Viel, J.F. (1998). Garlic, onion and cereal fibre as protective factors for breast cancer: a French case-control study. *European journal of epidemiology*, 14, 8, (Dec 1998), pp. (737-747), ISSN 0393-2990

Chambers, A.F. & Matrisian, L.M. (1997). Changing views of the role of matrix metalloproteinases in metastasis. *Journal of the National Cancer Institute*, 89, 17, (Sep 3 1997), pp. (1260-1270), ISSN 0027-8874

Charlier, E.; Conde, C.; Zhang, J.; Deneubourg, L.; Di Valentin, E.; Rahmouni, S.; Chariot, A.; Agostinis, P.; Pang, P.C.; Haslam, S.M.; Dell, A.; Penninger, J.; Erneux, C.; Piette, J. & Gloire, G. (2010). SHIP-1 inhibits CD95/APO-1/Fas-induced apoptosis in primary T lymphocytes and T leukemic cells by promoting CD95 glycosylation independently of its phosphatase activity. *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K*, 24, 4, (Apr 2010), pp. (821-832), ISSN 0887-6924

Chen, C.; Pung, D.; Leong, V.; Hebbar, V.; Shen, G.; Nair, S.; Li, W. & Kong, A.N. (2004). Induction of detoxifying enzymes by garlic organosulfur compounds through transcription factor Nrf2: effect of chemical structure and stress signals. *Free radical biology & medicine*, 37, 10, (Nov 15 2004), pp. (1578-1590), ISSN 0891-5849

Chen, D. & Zhou, Q. (2004). Caspase cleavage of BimEL triggers a positive feedback amplification of apoptotic signaling. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 5, (Feb 3 2004), pp. (1235-1240), ISSN 0027-8424

Chen, L.; Hong, J.Y.; So, E.; Hussin, A.H.; Cheng, W.F. & Yang, C.S. (1999). Decrease of hepatic catalase level by treatment with diallyl sulfide and garlic homogenates in rats and mice. *Journal of biochemical and molecular toxicology*, 13, 3-4, 1999), pp. (127-134), ISSN 1095-6670

Chiang, Y.H.; Jen, L.N.; Su, H.Y.; Lii, C.K.; Sheen, L.Y. & Liu, C.T. (2006). Effects of garlic oil and two of its major organosulfur compounds, diallyl disulfide and diallyl trisulfide, on intestinal damage in rats injected with endotoxin. *Toxicology and applied pharmacology*, 213, 1, (May 15 2006), pp. (46-54), ISSN 0041-008X

Chu, Q.; Ling, M.T.; Feng, H.; Cheung, H.W.; Tsao, S.W.; Wang, X. & Wong, Y.C. (2006). A novel anticancer effect of garlic derivatives: inhibition of cancer cell invasion through restoration of E-cadherin expression. *Carcinogenesis*, 27, 11, (Nov 2006), pp. (2180-2189), ISSN 0143-3334

Coppola, S. & Ghibelli, L. (2000). GSH extrusion and and the mitochondrial pathway of apoptotic signalling. *Biochemical Society transactions*, 28, 2, (Feb 2000), pp. (56-61), ISSN 0300-5127

Corzo-Martinez M., C.N., Villamiel M. (2007). Biological properties of onions and garlic. *Trends in Food Science & Technology*, 18, 2007), pp. (609-625), ISSN

Cox, M.C.; Low, J.; Lee, J.; Walshe, J.; Denduluri, N.; Berman, A.; Permenter, M.G.; Petros, W.P.; Price, D.K.; Figg, W.D.; Sparreboom, A. & Swain, S.M. (2006). Influence of garlic (Allium sativum) on the pharmacokinetics of docetaxel. *Clinical cancer research : an official journal of the American Association for Cancer Research*, 12, 15, (Aug 1 2006), pp. (4636-4640), ISSN 1078-0432

Das, A.; Banik, N.L. & Ray, S.K. (2007). Garlic compounds generate reactive oxygen species leading to activation of stress kinases and cysteine proteases for apoptosis in human glioblastoma T98G and U87MG cells. *Cancer*, 110, 5, (Sep 1 2007), pp. (1083-1095), ISSN 0008-543X

Davenport, D.M. & Wargovich, M.J. (2005). Modulation of cytochrome P450 enzymes by organosulfur compounds from garlic. *Food and chemical toxicology : an international*

journal published for the British Industrial Biological Research Association, 43, 12, (Dec 2005), pp. (1753-1762), ISSN 0278-6915

De Martino, A.; Filomeni, G.; Aquilano, K.; Ciriolo, M.R. & Rotilio, G. (2006). Effects of water garlic extracts on cell cycle and viability of HepG2 hepatoma cells. *The Journal of nutritional biochemistry*, 17, 11, (Nov 2006), pp. (742-749), ISSN 0955-2863

De Stefani, E.; Correa, P.; Boffetta, P.; Ronco, A.; Brennan, P.; Deneo-Pellegrini, H. & Mendilaharsu, M. (2001). Plant foods and risk of gastric cancer: a case-control study in Uruguay. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation*, 10, 4, (Aug 2001), pp. (357-364), ISSN 0959-8278

Dirsch, V.M.; Gerbes, A.L. & Vollmar, A.M. (1998). Ajoene, a compound of garlic, induces apoptosis in human promyeloleukemic cells, accompanied by generation of reactive oxygen species and activation of nuclear factor kappaB. *Molecular pharmacology*, 53, 3, (Mar 1998), pp. (402-407), ISSN 0026-895X

Dorant, E.; van den Brandt, P.A.; Goldbohm, R.A. & Sturmans, F. (1996). Consumption of onions and a reduced risk of stomach carcinoma. *Gastroenterology*, 110, 1, (Jan 1996), pp. (12-20), ISSN 0016-5085

Druesne, N.; Pagniez, A.; Mayeur, C.; Thomas, M.; Cherbuy, C.; Duee, P.H.; Martel, P. & Chaumontet, C. (2004). Diallyl disulfide (DADS) increases histone acetylation and p21(waf1/cip1) expression in human colon tumor cell lines. *Carcinogenesis*, 25, 7, (Jul 2004), pp. (1227-1236), ISSN 0143-3334

Druesne-Pecollo, N.; Chaumontet, C.; Pagniez, A.; Vaugelade, P.; Bruneau, A.; Thomas, M.; Cherbuy, C.; Duee, P.H. & Martel, P. (2007). In vivo treatment by diallyl disulfide increases histone acetylation in rat colonocytes. *Biochemical and biophysical research communications*, 354, 1, (Mar 2 2007), pp. (140-147), ISSN 0006-291X

Druesne-Pecollo, N. & Latino-Martel, P. (2011). Modulation of histone acetylation by garlic sulfur compounds. *Anti-cancer agents in medicinal chemistry*, 11, 3, (Mar 1 2011), pp. (254-259), ISSN 1871-5206

Egeblad, M. & Werb, Z. (2002). New functions for the matrix metalloproteinases in cancer progression. *Nature reviews. Cancer*, 2, 3, (Mar 2002), pp. (161-174), ISSN 1474-175X

Egen-Schwind, C.; Eckard, R.; Jekat, F.W. & Winterhoff, H. (1992a). Pharmacokinetics of vinyldithiins, transformation products of allicin. *Planta medica*, 58, 1, (Feb 1992a), pp. (8-13), ISSN 0032-0943

Egen-Schwind, C.; Eckard, R. & Kemper, F.H. (1992b). Metabolism of garlic constituents in the isolated perfused rat liver. *Planta medica*, 58, 4, (Aug 1992b), pp. (301-305), ISSN 0032-0943

Ellerbroek, S.M. & Stack, M.S. (1999). Membrane associated matrix metalloproteinases in metastasis. *BioEssays : news and reviews in molecular, cellular and developmental biology*, 21, 11, (Nov 1999), pp. (940-949), ISSN 0265-9247

Espino, P.S.; Drobic, B.; Dunn, K.L. & Davie, J.R. (2005). Histone modifications as a platform for cancer therapy. *Journal of cellular biochemistry*, 94, 6, (Apr 15 2005), pp. (1088-1102), ISSN 0730-2312

Filomeni, G.; Aquilano, K.; Rotilio, G. & Ciriolo, M.R. (2003). Reactive oxygen species-dependent c-Jun NH2-terminal kinase/c-Jun signaling cascade mediates neuroblastoma cell death induced by diallyl disulfide. *Cancer research*, 63, 18, (Sep 15 2003), pp. (5940-5949), ISSN 0008-5472

Fisher, C.D.; Augustine, L.M.; Maher, J.M.; Nelson, D.M.; Slitt, A.L.; Klaassen, C.D.; Lehman-McKeeman, L.D. & Cherrington, N.J. (2007). Induction of drug-metabolizing enzymes by garlic and allyl sulfide compounds via activation of constitutive androstane receptor and nuclear factor E2-related factor 2. *Drug metabolism and disposition: the biological fate of chemicals*, 35, 6, (Jun 2007), pp. (995-1000), ISSN 0090-9556

Fleischauer, A.T.; Poole, C. & Arab, L. (2000). Garlic consumption and cancer prevention: meta-analyses of colorectal and stomach cancers. *The American journal of clinical nutrition*, 72, 4, (Oct 2000), pp. (1047-1052), ISSN 0002-9165

Fleischauer, A.T. & Arab, L. (2001). Garlic and cancer: a critical review of the epidemiologic literature. *The Journal of nutrition*, 131, 3s, (Mar 2001), pp. (1032S-1040S), ISSN 0022-3166

Freeman F., K.Y. (1995). Garlic chemistry: Stability of S-(2-propenyl)-2-propene-1-sulfinothionate (allicin) in blood, solvents, and simulated physiological fluids. *Journal of agricultural and food chemistry*, 43, 9, 1995), pp. (2332-2338), ISSN

Fukao, T.; Hosono, T.; Misawa, S.; Seki, T. & Ariga, T. (2004). The effects of allyl sulfides on the induction of phase II detoxification enzymes and liver injury by carbon tetrachloride. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, 42, 5, (May 2004), pp. (743-749), ISSN 0278-6915

Galeone, C.; Pelucchi, C.; Levi, F.; Negri, E.; Franceschi, S.; Talamini, R.; Giacosa, A. & La Vecchia, C. (2006). Onion and garlic use and human cancer. *The American journal of clinical nutrition*, 84, 5, (Nov 2006), pp. (1027-1032), ISSN 0002-9165

Galeone, C.; Pelucchi, C.; Dal Maso, L.; Negri, E.; Montella, M.; Zucchetto, A.; Talamini, R. & La Vecchia, C. (2009). Allium vegetables intake and endometrial cancer risk. *Public health nutrition*, 12, 9, (Sep 2009), pp. (1576-1579), ISSN 1368-9800

Gao, C.M.; Takezaki, T.; Ding, J.H.; Li, M.S. & Tajima, K. (1999). Protective effect of allium vegetables against both esophageal and stomach cancer: a simultaneous case-referent study of a high-epidemic area in Jiangsu Province, China. *Japanese journal of cancer research : Gann*, 90, 6, (Jun 1999), pp. (614-621), ISSN 0910-5050

Gapter, L.A.; Yuin, O.Z. & Ng, K.Y. (2008). S-Allylcysteine reduces breast tumor cell adhesion and invasion. *Biochemical and biophysical research communications*, 367, 2, (Mar 7 2008), pp. (446-451), ISSN 0006-291X

Geng, Z., Lau B.H.S. (1997). Aged garlic extracts modulate glutathione redox cycle and superoxide dismutase activity in vascular endothelial cells. *Phytotherapy research : PTR*, 11, 1997), pp. (54-56), ISSN

Germain, E.; Auger, J.; Ginies, C.; Siess, M.H. & Teyssier, C. (2002). In vivo metabolism of diallyl disulphide in the rat: identification of two new metabolites. *Xenobiotica; the fate of foreign compounds in biological systems*, 32, 12, (Dec 2002), pp. (1127-1138), ISSN 0049-8254

Gomez, D.E.; Alonso, D.F.; Yoshiji, H. & Thorgeirsson, U.P. (1997). Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *European journal of cell biology*, 74, 2, (Oct 1997), pp. (111-122), ISSN 0171-9335

Goncagul, G. & Ayaz, E. (2010). Antimicrobial effect of garlic (Allium sativum). *Recent patents on anti-infective drug discovery*, 5, 1, (Jan 1 2010), pp. (91-93), ISSN 1574-891X

Guo, Z.; Müller, D.; Pentz, R.; Kress, G. & Siegers, C.P. (1990). Bioavailability of sulphurcontaining ingredients of garlic in the rat. *Planta medica*, 56, 1990), pp. (692-699), ISSN

Hassan, H.T. (2004). Ajoene (natural garlic compound): a new anti-leukaemia agent for AML therapy. *Leukemia research*, 28, 7, (Jul 2004), pp. (667-671), ISSN 0145-2126

Herman-Antosiewicz, A. & Singh, S.V. (2005). Checkpoint kinase 1 regulates diallyl trisulfide-induced mitotic arrest in human prostate cancer cells. *The Journal of biological chemistry*, 280, 31, (Aug 5 2005), pp. (28519-28528), ISSN 0021-9258

Herman-Antosiewicz, A.; Powolny, A.A. & Singh, S.V. (2007a). Molecular targets of cancer chemoprevention by garlic-derived organosulfides. *Acta pharmacologica Sinica*, 28, 9, (Sep 2007a), pp. (1355-1364), ISSN 1671-4083

Herman-Antosiewicz, A.; Stan, S.D.; Hahm, E.R.; Xiao, D. & Singh, S.V. (2007b). Activation of a novel ataxia-telangiectasia mutated and Rad3 related/checkpoint kinase 1-dependent prometaphase checkpoint in cancer cells by diallyl trisulfide, a promising cancer chemopreventive constituent of processed garlic. *Molecular cancer therapeutics*, 6, 4, (Apr 2007b), pp. (1249-1261), ISSN 1535-7163

Herman-Antosiewicz, A.; Kim, Y.A.; Kim, S.H.; Xiao, D. & Singh, S.V. (2010). Diallyl trisulfide-induced G2/M phase cell cycle arrest in DU145 cells is associated with delayed nuclear translocation of cyclin-dependent kinase 1. *Pharmaceutical research*, 27, 6, (Jun 2010), pp. (1072-1079), ISSN 0724-8741

Hirohata, T. & Kono, S. (1997). Diet/nutrition and stomach cancer in Japan. *International journal of cancer. Journal international du cancer*, Suppl 10, 1997), pp. (34-36), ISSN 0020-7136

Ho, B.E.; Shen, D.D.; McCune, J.S.; Bui, T.; Risler, L.; Yang, Z. & Ho, R.J. (2010). Effects of Garlic on Cytochromes P450 2C9- and 3A4-Mediated Drug Metabolism in Human Hepatocytes. *Scientia pharmaceutica*, 78, 3, (Oct 30 2010), pp. (473-481), ISSN 0036-8709

Hong, Y.S.; Ham, Y.A.; Choi, J.H. & Kim, J. (2000). Effects of allyl sulfur compounds and garlic extract on the expression of Bcl-2, Bax, and p53 in non small cell lung cancer cell lines. *Experimental & molecular medicine*, 32, 3, (Sep 30 2000), pp. (127-134), ISSN 1226-3613

Horie, T.; Murayama, T.; Mishima, T.; Itoh, F.; Minamide, Y.; Fuwa, T. & Awazu, S. (1989). Protection of liver microsomal membranes from lipid peroxidation by garlic extract. *Planta medica*, 55, 6, (Dec 1989), pp. (506-508), ISSN 0032-0943

Hosono, T.; Fukao, T.; Ogihara, J.; Ito, Y.; Shiba, H.; Seki, T. & Ariga, T. (2005). Diallyl trisulfide suppresses the proliferation and induces apoptosis of human colon cancer cells through oxidative modification of beta-tubulin. *The Journal of biological chemistry*, 280, 50, (Dec 16 2005), pp. (41487-41493), ISSN 0021-9258

Hosono, T.; Hosono-Fukao, T.; Inada, K.; Tanaka, R.; Yamada, H.; Iitsuka, Y.; Seki, T.; Hasegawa, I. & Ariga, T. (2008). Alkenyl group is responsible for the disruption of microtubule network formation in human colon cancer cell line HT-29 cells. *Carcinogenesis*, 29, 7, (Jul 2008), pp. (1400-1406), ISSN 0143-3334

Howard, E.W.; Ling, M.T.; Chua, C.W.; Cheung, H.W.; Wang, X. & Wong, Y.C. (2007). Garlic-derived S-allylmercaptocysteine is a novel in vivo antimetastatic agent for androgen-independent prostate cancer. *Clinical cancer research : an official journal of*

the American Association for Cancer Research, 13, 6, (Mar 15 2007), pp. (1847-1856), ISSN 1078-0432

Howard, E.W.; Lee, D.T.; Chiu, Y.T.; Chua, C.W.; Wang, X. & Wong, Y.C. (2008). Evidence of a novel docetaxel sensitizer, garlic-derived S-allylmercaptocysteine, as a treatment option for hormone refractory prostate cancer. *International journal of cancer. Journal international du cancer*, 122, 9, (May 1 2008), pp. (1941-1948), ISSN 0020-7136

Hsing, A.W.; Chokkalingam, A.P.; Gao, Y.T.; Madigan, M.P.; Deng, J.; Gridley, G. & Fraumeni, J.F., Jr. (2002). Allium vegetables and risk of prostate cancer: a population-based study. *Journal of the National Cancer Institute*, 94, 21, (Nov 6 2002), pp. (1648-1651), ISSN 0027-8874

Hu, X.; Benson, P.J.; Srivastava, S.K.; Mack, L.M.; Xia, H.; Gupta, V.; Zaren, H.A. & Singh, S.V. (1996). Glutathione S-transferases of female A/J mouse liver and forestomach and their differential induction by anti-carcinogenic organosulfides from garlic. *Archives of biochemistry and biophysics*, 336, 2, (Dec 15 1996), pp. (199-214), ISSN 0003-9861

Hu, X.; Cao, B.N.; Hu, G.; He, J.; Yang, D.Q. & Wan, Y.S. (2002). Attenuation of cell migration and induction of cell death by aged garlic extract in rat sarcoma cells. *International journal of molecular medicine*, 9, 6, (Jun 2002), pp. (641-643), ISSN 1107-3756

Iciek, M.; Kwiecien, I. & Wlodek, L. (2009). Biological properties of garlic and garlic-derived organosulfur compounds. *Environmental and molecular mutagenesis*, 50, 3, (Apr 2009), pp. (247-265), ISSN 0893-6692

Imai, J.; Ide, N.; Nagae, S.; Moriguchi, T.; Matsuura, H. & Itakura, Y. (1994). Antioxidant and radical scavenging effects of aged garlic extract and its constituents. *Planta medica*, 60, 5, (Oct 1994), pp. (417-420), ISSN 0032-0943

Jacob, C. (2006). A scent of therapy: pharmacological implications of natural products containing redox-active sulfur atoms. *Natural product reports*, 23, 6, (Dec 2006), pp. (851-863), ISSN 0265-0568

Jandke, J. & Spiteller, G. (1987). Unusual conjugates in biological profiles originating from consumption of onions and garlic. *Journal of chromatography*, 421, 1, (Oct 9 1987), pp. (1-8), ISSN 0021-9673

Jin, L. & Baillie, T.A. (1997). Metabolism of the chemoprotective agent diallyl sulfide to glutathione conjugates in rats. *Chemical research in toxicology*, 10, 3, (Mar 1997), pp. (318-327), ISSN 0893-228X

Jirousek L., J.J. (1956). Antifungal properties of organic polysulphides from cabbage. *Die Naturwissenschaften*, 43, 16, 1956), pp. (375-376), ISSN

Jo, H.J.; Song, J.D.; Kim, K.M.; Cho, Y.H.; Kim, K.H. & Park, Y.C. (2008). Diallyl disulfide induces reversible G2/M phase arrest on a p53-independent mechanism in human colon cancer HCT-116 cells. *Oncology reports*, 19, 1, (Jan 2008), pp. (275-280), ISSN 1021-335X

Jordan, A.; Hadfield, J.A.; Lawrence, N.J. & McGown, A.T. (1998). Tubulin as a target for anticancer drugs: agents which interact with the mitotic spindle. *Medicinal research reviews*, 18, 4, (Jul 1998), pp. (259-296), ISSN 0198-6325

Kalra, N.; Arora, A. & Shukla, Y. (2006). Involvement of multiple signaling pathways in diallyl sulfide mediated apoptosis in mouse skin tumors. *Asian Pacific journal of cancer prevention : APJCP*, 7, 4, (Oct-Dec 2006), pp. (556-562), ISSN 1513-7368

Kamel A., S.M. (2000). Recent studies on the chemistry and biological activities of the organosulfur compounds of garlic (Allium sativum). *Studies in Natural Products Chemistry*, 23, 2000), pp. (455-485), ISSN

Karin, M. & Greten, F.R. (2005). NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nature reviews. Immunology*, 5, 10, (Oct 2005), pp. (749-759), ISSN 1474-1733

Karmakar, S.; Banik, N.L.; Patel, S.J. & Ray, S.K. (2007). Garlic compounds induced calpain and intrinsic caspase cascade for apoptosis in human malignant neuroblastoma SH-SY5Y cells. *Apoptosis : an international journal on programmed cell death*, 12, 4, (Apr 2007), pp. (671-684), ISSN 1360-8185

Kessenbrock, K.; Plaks, V. & Werb, Z. (2010). Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell*, 141, 1, (Apr 2 2010), pp. (52-67), ISSN 0092-8674

Kim, J.Y. & Kwon, O. (2009). Garlic intake and cancer risk: an analysis using the Food and Drug Administration's evidence-based review system for the scientific evaluation of health claims. *The American journal of clinical nutrition*, 89, 1, (Jan 2009), pp. (257-264), ISSN 0002-9165

Kim, Y.A.; Xiao, D.; Xiao, H.; Powolny, A.A.; Lew, K.L.; Reilly, M.L.; Zeng, Y.; Wang, Z. & Singh, S.V. (2007). Mitochondria-mediated apoptosis by diallyl trisulfide in human prostate cancer cells is associated with generation of reactive oxygen species and regulated by Bax/Bak. *Molecular cancer therapeutics*, 6, 5, (May 2007), pp. (1599-1609), ISSN 1535-7163

Knowles, L.M. & Milner, J.A. (1998). Depressed p34cdc2 kinase activity and G2/M phase arrest induced by diallyl disulfide in HCT-15 cells. *Nutrition and cancer*, 30, 3, 1998), pp. (169-174), ISSN 0163-5581

Knowles, L.M. & Milner, J.A. (2000). Diallyl disulfide inhibits p34(cdc2) kinase activity through changes in complex formation and phosphorylation. *Carcinogenesis*, 21, 6, (Jun 2000), pp. (1129-1134), ISSN 0143-3334

Kris-Etherton, P.M.; Hecker, K.D.; Bonanome, A.; Coval, S.M.; Binkoski, A.E.; Hilpert, K.F.; Griel, A.E. & Etherton, T.D. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American journal of medicine*, 113 Suppl 9B, (Dec 30 2002), pp. (71S-88S), ISSN 0002-9343

Kundu, J.K. & Surh, Y.J. (2008). Inflammation: gearing the journey to cancer. *Mutation research*, 659, 1-2, (Jul-Aug 2008), pp. (15-30), ISSN 0027-5107

Kwon, K.B.; Yoo, S.J.; Ryu, D.G.; Yang, J.Y.; Rho, H.W.; Kim, J.S.; Park, J.W.; Kim, H.R. & Park, B.H. (2002). Induction of apoptosis by diallyl disulfide through activation of caspase-3 in human leukemia HL-60 cells. *Biochemical pharmacology*, 63, 1, (Jan 1 2002), pp. (41-47), ISSN 0006-2952

Lai, K.C.; Hsu, S.C.; Kuo, C.L.; Yang, J.S.; Ma, C.Y.; Lu, H.F.; Tang, N.Y.; Hsia, T.C.; Ho, H.C. & Chung, J.G. (2011). Diallyl sulfide, diallyl disulfide, and diallyl trisulfide inhibit migration and invasion in human colon cancer colo 205 cells through the inhibition of matrix metalloproteinase-2, -7, and -9 expressions. *Environmental toxicology*, (Jun 21 2011), pp. ISSN 1520-4081

Lamm, D.L. & Riggs, D.R. (2001). Enhanced immunocompetence by garlic: role in bladder cancer and other malignancies. *The Journal of nutrition*, 131, 3s, (Mar 2001), pp. (1067S-1070S), ISSN 0022-3166

Lanzotti, V. (2006). The analysis of onion and garlic. *Journal of chromatography. A*, 1112, 1-2, (Apr 21 2006), pp. (3-22), ISSN 0021-9673

Lea, M.A.; Randolph, V.M. & Patel, M. (1999). Increased acetylation of histones induced by diallyl disulfide and structurally related molecules. *International journal of oncology*, 15, 2, (Aug 1999), pp. (347-352), ISSN 1019-6439

Lea, M.A. & Randolph, V.M. (2001). Induction of histone acetylation in rat liver and hepatoma by organosulfur compounds including diallyl disulfide. *Anticancer research*, 21, 4A, (Jul-Aug 2001), pp. (2841-2845), ISSN 0250-7005

Lea, M.A.; Rasheed, M.; Randolph, V.M.; Khan, F.; Shareef, A. & desBordes, C. (2002). Induction of histone acetylation and inhibition of growth of mouse erythroleukemia cells by S-allylmercaptocysteine. *Nutrition and cancer*, 43, 1, 2002), pp. (90-102), ISSN 0163-5581

Lee, Y. (2008). Induction of apoptosis by S-allylmercapto-L-cysteine, a biotransformed garlic derivative, on a human gastric cancer cell line. *International journal of molecular medicine*, 21, 6, (Jun 2008), pp. (765-770), ISSN 1107-3756

Lei, X.Y.; Yao, S.Q.; Zu, X.Y.; Huang, Z.X.; Liu, L.J.; Zhong, M.; Zhu, B.Y.; Tang, S.S. & Liao, D.F. (2008). Apoptosis induced by diallyl disulfide in human breast cancer cell line MCF-7. *Acta pharmacologica Sinica*, 29, 10, (Oct 2008), pp. (1233-1239), ISSN 1671-4083

Li, M.; Ciu, J.R.; Ye, Y.; Min, J.M.; Zhang, L.H.; Wang, K.; Gares, M.; Cros, J.; Wright, M. & Leung-Tack, J. (2002a). Antitumor activity of Z-ajoene, a natural compound purified from garlic: antimitotic and microtubule-interaction properties. *Carcinogenesis*, 23, 4, (Apr 2002a), pp. (573-579), ISSN 0143-3334

Li, M.; Min, J.M.; Cui, J.R.; Zhang, L.H.; Wang, K.; Valette, A.; Davrinche, C.; Wright, M. & Leung-Tack, J. (2002b). Z-ajoene induces apoptosis of HL-60 cells: involvement of Bcl-2 cleavage. *Nutrition and cancer*, 42, 2, 2002b), pp. (241-247), ISSN 0163-5581

Ling, H.; Zhang, L.Y.; Su, Q.; Song, Y.; Luo, Z.Y.; Zhou, X.T.; Zeng, X.; He, J.; Tan, H. & Yuan, J.P. (2006). Erk is involved in the differentiation induced by diallyl disulfide in the human gastric cancer cell line MGC803. *Cellular & molecular biology letters*, 11, 3, 2006), pp. (408-423), ISSN 1425-8153

Lu, H.F.; Sue, C.C.; Yu, C.S.; Chen, S.C.; Chen, G.W. & Chung, J.G. (2004). Diallyl disulfide (DADS) induced apoptosis undergo caspase-3 activity in human bladder cancer T24 cells. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, 42, 10, (Oct 2004), pp. (1543-1552), ISSN 0278-6915

Lund, T.; Stokke, T.; Olsen, O.E. & Fodstad, O. (2005). Garlic arrests MDA-MB-435 cancer cells in mitosis, phosphorylates the proapoptotic BH3-only protein BimEL and induces apoptosis. *British journal of cancer*, 92, 9, (May 9 2005), pp. (1773-1781), ISSN 0007-0920

Luttun, A.; Dewerchin, M.; Collen, D. & Carmeliet, P. (2000). The role of proteinases in angiogenesis, heart development, restenosis, atherosclerosis, myocardial ischemia, and stroke: insights from genetic studies. *Current atherosclerosis reports*, 2, 5, (Sep 2000), pp. (407-416), ISSN 1523-3804

Makrilia, N.; Lappa, T.; Xyla, V.; Nikolaidis, I. & Syrigos, K. (2009). The role of angiogenesis in solid tumours: an overview. *European journal of internal medicine*, 20, 7, (Nov 2009), pp. (663-671), ISSN 0953-6205

Malki, A.; El-Saadani, M. & Sultan, A.S. (2009). Garlic constituent diallyl trisulfide induced apoptosis in MCF7 human breast cancer cells. *Cancer biology & therapy*, 8, 22, (Nov 2009), pp. (2175-2185), ISSN 1538-4047

Matsuura, N.; Miyamae, Y.; Yamane, K.; Nagao, Y.; Hamada, Y.; Kawaguchi, N.; Katsuki, T.; Hirata, K.; Sumi, S. & Ishikawa, H. (2006). Aged garlic extract inhibits angiogenesis and proliferation of colorectal carcinoma cells. *The Journal of nutrition*, 136, 3 Suppl, (Mar 2006), pp. (842S-846S), ISSN 0022-3166

Melino, S.; Sabelli, R. & Paci, M. (2011). Allyl sulfur compounds and cellular detoxification system: effects and perspectives in cancer therapy. *Amino acids*, 41, 1, (Jun 2011), pp. (103-112), ISSN 0939-4451

Meyer, K.; Ueberham, E. & Gebhardt, R. (2004). Influence of organosulphur compounds from garlic on the secretion of matrix metalloproteinases and their inhibitor TIMP-1 by cultured HUVEC cells. *Cell biology and toxicology*, 20, 4, (Jul 2004), pp. (253-260), ISSN 0742-2091

Miean, K.H. & Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of agricultural and food chemistry*, 49, 6, (Jun 2001), pp. (3106-3112), ISSN 0021-8561

Mollinedo, F. & Gajate, C. (2003). Microtubules, microtubule-interfering agents and apoptosis. *Apoptosis : an international journal on programmed cell death*, 8, 5, (Oct 2003), pp. (413-450), ISSN 1360-8185

Munchberg, U.; Anwar, A.; Mecklenburg, S. & Jacob, C. (2007). Polysulfides as biologically active ingredients of garlic. *Organic & biomolecular chemistry*, 5, 10, (May 21 2007), pp. (1505-1518), ISSN 1477-0520

Munday, R.; Munday, J.S. & Munday, C.M. (2003). Comparative effects of mono-, di-, tri-, and tetrasulfides derived from plants of the Allium family: redox cycling in vitro and hemolytic activity and Phase 2 enzyme induction in vivo. *Free radical biology & medicine*, 34, 9, (May 1 2003), pp. (1200-1211), ISSN 0891-5849

Munoz, N.; Plummer, M.; Vivas, J.; Moreno, V.; De Sanjose, S.; Lopez, G. & Oliver, W. (2001). A case-control study of gastric cancer in Venezuela. *International journal of cancer. Journal international du cancer*, 93, 3, (Aug 1 2001), pp. (417-423), ISSN 0020-7136

Nagae, S.; Ushijima, M.; Hatono, S.; Imai, J.; Kasuga, S.; Matsuura, H.; Itakura, Y. & Higashi, Y. (1994). Pharmacokinetics of the garlic compound S-allylcysteine. *Planta medica*, 60, 3, (Jun 1994), pp. (214-217), ISSN 0032-0943

Nagase, H. & Woessner, J.F., Jr. (1999). Matrix metalloproteinases. *The Journal of biological chemistry*, 274, 31, (Jul 30 1999), pp. (21491-21494), ISSN 0021-9258

Nakagawa, H.; Tsuta, K.; Kiuchi, K.; Senzaki, H.; Tanaka, K.; Hioki, K. & Tsubura, A. (2001). Growth inhibitory effects of diallyl disulfide on human breast cancer cell lines. *Carcinogenesis*, 22, 6, (Jun 2001), pp. (891-897), ISSN 0143-3334

Nencini, C.; Cavallo, F.; Capasso, A.; Franchi, G.G.; Giorgio, G. & Micheli, L. (2007). Evaluation of antioxidative properties of Allium species growing wild in Italy. *Phytotherapy research : PTR*, 21, 9, (Sep 2007), pp. (874-878), ISSN 0951-418X

Nian, H.; Delage, B.; Pinto, J.T. & Dashwood, R.H. (2008). Allyl mercaptan, a garlic-derived organosulfur compound, inhibits histone deacetylase and enhances Sp3 binding on the P21WAF1 promoter. *Carcinogenesis*, 29, 9, (Sep 2008), pp. (1816-1824), ISSN 0143-3334

Nian, H.; Delage, B.; Ho, E. & Dashwood, R.H. (2009). Modulation of histone deacetylase activity by dietary isothiocyanates and allyl sulfides: studies with sulforaphane and garlic organosulfur compounds. *Environmental and molecular mutagenesis*, 50, 3, (Apr 2009), pp. (213-221), ISSN 0893-6692

Nishimura, H.; Takahashi, T.; Wijaya, C.H.; Satoh, A. & Ariga, T. (2000). Thermochemical transformation of sulfur compounds in Japanese domestic Allium, Allium victorialis L. *BioFactors*, 13, 1-4, 2000), pp. (257-263), ISSN 0951-6433

Nishimura, H.; Higuchi, O. & Tateshita, K. (2004). Antioxidative activity of sulfur-containing compounds in Allium species for human LDL oxidation in vitro. *BioFactors*, 21, 1-4, 2004), pp. (277-280), ISSN 0951-6433

Okada, Y.; Tanaka, K.; Fujita, I.; Sato, E. & Okajima, H. (2005). Antioxidant activity of thiosulfinates derived from garlic. *Redox report : communications in free radical research*, 10, 2, 2005), pp. (96-102), ISSN 1351-0002

Park, K.A.; Kweon, S. & Choi, H. (2002). Anticarcinogenic effect and modification of cytochrome P450 2E1 by dietary garlic powder in diethylnitrosamine-initiated rat hepatocarcinogenesis. *Journal of biochemistry and molecular biology*, 35, 6, (Nov 30 2002), pp. (615-622), ISSN 1225-8687

Pelucchi, C.; Bosetti, C.; Rossi, M.; Negri, E. & La Vecchia, C. (2009). Selected aspects of Mediterranean diet and cancer risk. *Nutrition and cancer*, 61, 6, 2009), pp. (756-766), ISSN 0163-5581

Peng, C.W.; Liu, X.L.; Liu, X. & Li, Y. (2010). Co-evolution of cancer microenvironment reveals distinctive patterns of gastric cancer invasion: laboratory evidence and clinical significance. *Journal of translational medicine*, 8, 2010), pp. (101), ISSN 1479-5876

Polette, M. & Birembaut, P. (1998). Membrane-type metalloproteinases in tumor invasion. *The international journal of biochemistry & cell biology*, 30, 11, (Nov 1998), pp. (1195-1202), ISSN 1357-2725

Powolny, A.A. & Singh, S.V. (2008). Multitargeted prevention and therapy of cancer by diallyl trisulfide and related Allium vegetable-derived organosulfur compounds. *Cancer letters*, 269, 2, (Oct 8 2008), pp. (305-314), ISSN 0304-3835

Prager-Khoutorsky, M.; Goncharov, I.; Rabinkov, A.; Mirelman, D.; Geiger, B. & Bershadsky, A.D. (2007). Allicin inhibits cell polarization, migration and division via its direct effect on microtubules. *Cell motility and the cytoskeleton*, 64, 5, (May 2007), pp. (321-337), ISSN 0886-1544

Prasad, S.; Kalra, N.; Srivastava, S. & Shukla, Y. (2008). Regulation of oxidative stress-mediated apoptosis by diallyl sulfide in DMBA-exposed Swiss mice. *Human & experimental toxicology*, 27, 1, (Jan 2008), pp. (55-63), ISSN 0960-3271

Pratheeshkumar, P.; Thejass, P. & Kutan, G. (2010). Diallyl disulfide induces caspase-dependent apoptosis via mitochondria-mediated intrinsic pathway in B16F-10 melanoma cells by up-regulating p53, caspase-3 and down-regulating pro-inflammatory cytokines and nuclear factor-kappabeta-mediated Bcl-2 activation. *Journal of environmental pathology, toxicology and oncology : official organ of the International Society for Environmental Toxicology and Cancer*, 29, 2, 2010), pp. (113-125), ISSN 0731-8898

Pushpendran, C.K.; Devasagayam, T.P.; Chintalwar, G.J.; Banerji, A. & Eapen, J. (1980). The metabolic fate of [35S]-diallyl disulphide in mice. *Experientia*, 36, 8, (Aug 15 1980), pp. (1000-1001), ISSN 0014-4754

Rabinkov, A.; Miron, T.; Konstantinovski, L.; Wilchek, M.; Mirelman, D. & Weiner, L. (1998). The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. *Biochimica et biophysica acta*, 1379, 2, (Feb 2 1998), pp. (233-244), ISSN 0006-3002

Rahman, K. & Lowe, G.M. (2006). Garlic and cardiovascular disease: a critical review. *The Journal of nutrition*, 136, 3 Suppl, (Mar 2006), pp. (736S-740S), ISSN 0022-3166

Razo-Rodriguez, A.C.; Chirino, Y.I.; Sanchez-Gonzalez, D.J.; Martinez-Martinez, C.M.; Cruz, C. & Pedraza-Chaverri, J. (2008). Garlic powder ameliorates cisplatin-induced nephrotoxicity and oxidative stress. *Journal of medicinal food*, 11, 3, (Sep 2008), pp. (582-586), ISSN 1096-620X

Rosen, R.T.; Hiserodt, R.D.; Fukuda, E.K.; Ruiz, R.J.; Zhou, Z.; Lech, J.; Rosen, S.L. & Hartman, T.G. (2001). Determination of allicin, S-allylcysteine and volatile metabolites of garlic in breath, plasma or simulated gastric fluids. *The Journal of nutrition*, 131, 3s, (Mar 2001), pp. (968S-971S), ISSN 0022-3166

Salem, S.; Salahi, M.; Mohseni, M.; Ahmadi, H.; Mehrsai, A.; Jahani, Y. & Pourmand, G. (2011). Major dietary factors and prostate cancer risk: a prospective multicenter case-control study. *Nutrition and cancer*, 63, 1, (Jan 2011), pp. (21-27), ISSN 0163-5581

Sancar, A.; Lindsey-Boltz, L.A.; Unsal-Kacmaz, K. & Linn, S. (2004). Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annual review of biochemistry*, 73, 2004), pp. (39-85), ISSN 0066-4154

Satia, J.A.; Littman, A.; Slatore, C.G.; Galanko, J.A. & White, E. (2009). Associations of herbal and specialty supplements with lung and colorectal cancer risk in the VITamins and Lifestyle study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*, 18, 5, (May 2009), pp. (1419-1428), ISSN 1055-9965

Scharfenberg, K.; Ryll, T.; Wagner, R. & Wagner, K.G. (1994). Injuries to cultivated BJA-B cells by ajoene, a garlic-derived natural compound: cell viability, glutathione metabolism, and pools of acidic amino acids. *Journal of cellular physiology*, 158, 1, (Jan 1994), pp. (55-60), ISSN 0021-9541

Scherer, C.; Jacob, C.; Dicato, M. & Diederich, M. (2009). Potential role of organic sulfur compounds from Allium species in cancer prevention and therapy. *Phytochem. Rev.*, 8, 2009), pp. (349-368), ISSN

Scherer, C.; Jacob, C.; Dicato, M. & Diederich, M. (2010). Allylsulfides as Bioactive Compounds with Chempretherapeutic and/or Chemopreventive Effects, In: *Comprehensive Bioactive Natural Products*, S. Press, pp. ISBN : 1-933699-50-7, New Dheli, India

Schneider, T.; Ba, L.A.; Khairan, K.; Zwergel, C.; Bach, N.D.; Bernhardt, I.; Brandt, W.; Weissjohann, L.; Diederich, M. & Jacob, C. (2011a). Interactions of polysulfanes with components of red blood cells. *MedChemCommun*, 2, 3, (Mar 2011a), pp. (196-200), ISSN 2040-2503

Schneider, T.; Baldauf, A.; Ba, L.A.; Jamier, V.; Khairan, K.; Sarakbi, M.B.; Reum, N.; Schneider, M.; Röseler, A.; Becker, K.; Burkholz, T.; Winyard, P.G.; Kelkel, M.;

Diederich, M. & Jacob, C. (2011b). Selective Antimicrobial Activity Associated with Sulfur Nanoparticles. *Journal of Biomedical Nanotechnology*, 7, 2, (Jun 2011b), pp. (1-11), ISSN 1550-7033

Seki, T.; Tsuji, K.; Hayato, Y.; Moritomo, T. & Ariga, T. (2000). Garlic and onion oils inhibit proliferation and induce differentiation of HL-60 cells. *Cancer letters*, 160, 1, (Nov 10 2000), pp. (29-35), ISSN 0304-3835

Sengupta, A.; Ghosh, S. & Das, S. (2003). Tomato and garlic can modulate azoxymethane-induced colon carcinogenesis in rats. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation*, 12, 3, (Jun 2003), pp. (195-200), ISSN 0959-8278

Sengupta, A.; Ghosh, S.; Bhattacharjee, S. & Das, S. (2004a). Indian food ingredients and cancer prevention - an experimental evaluation of anticarcinogenic effects of garlic in rat colon. *Asian Pacific journal of cancer prevention : APJCP*, 5, 2, (Apr-Jun 2004a), pp. (126-132), ISSN 1513-7368

Sengupta, A.; Ghosh, S. & Das, S. (2004b). Modulatory influence of garlic and tomato on cyclooxygenase-2 activity, cell proliferation and apoptosis during azoxymethane induced colon carcinogenesis in rat. *Cancer letters*, 208, 2, (May 28 2004b), pp. (127-136), ISSN 0304-3835

Shen, Y. & White, E. (2001). p53-dependent apoptosis pathways. *Advances in cancer research*, 82, 2001), pp. (55-84), ISSN 0065-230X

Sheweita, S.A. (2000). Drug-metabolizing enzymes: mechanisms and functions. *Current drug metabolism*, 1, 2, (Sep 2000), pp. (107-132), ISSN 1389-2002

Sheweita, S.A. & Tilmisany, A.K. (2003). Cancer and phase II drug-metabolizing enzymes. *Current drug metabolism*, 4, 1, (Feb 2003), pp. (45-58), ISSN 1389-2002

Shimada, T. (2006). Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. *Drug metabolism and pharmacokinetics*, 21, 4, (Aug 2006), pp. (257-276), ISSN 1347-4367

Shin, D.Y.; Kim, G.Y.; Kim, J.I.; Yoon, M.K.; Kwon, T.K.; Lee, S.J.; Choi, Y.W.; Kang, H.S.; Yoo, Y.H. & Choi, Y.H. (2010). Anti-invasive activity of diallyl disulfide through tightening of tight junctions and inhibition of matrix metalloproteinase activities in LNCaP prostate cancer cells. *Toxicology in vitro : an international journal published in association with BIBRA*, 24, 6, (Sep 2010), pp. (1569-1576), ISSN 0887-2333

Singh, S.V.; Mack, L.M.; Xia, H.; Gupta, V. & al., e. (1997). Differential induction of glutathione redox-cycle enzymes by anti-carcinogenic organosulfides from garlic. *Clin Chem Enzym Commun*, 7, 1997), pp. (287–297), ISSN

Singh, S.V.; Powolny, A.A.; Stan, S.D.; Xiao, D.; Arlotti, J.A.; Warin, R.; Hahm, E.R.; Marynowski, S.W.; Bommareddy, A.; Potter, D.M. & Dhir, R. (2008). Garlic constituent diallyl trisulfide prevents development of poorly differentiated prostate cancer and pulmonary metastasis multiplicity in TRAMP mice. *Cancer research*, 68, 22, (Nov 15 2008), pp. (9503-9511), ISSN 0008-5472

Song, J.D.; Lee, S.K.; Kim, K.M.; Park, S.E.; Park, S.J.; Kim, K.H.; Ahn, S.C. & Park, Y.C. (2009). Molecular mechanism of diallyl disulfide in cell cycle arrest and apoptosis in HCT-116 colon cancer cells. *Journal of biochemical and molecular toxicology*, 23, 1, (Jan-Feb 2009), pp. (71-79), ISSN 1095-6670

Sriram, N.; Kalayarasan, S.; Ashokkumar, P.; Sureshkumar, A. & Sudhandiran, G. (2008). Diallyl sulfide induces apoptosis in Colo 320 DM human colon cancer cells:

involvement of caspase-3, NF-kappaB, and ERK-2. *Molecular and cellular biochemistry*, 311, 1-2, (Apr 2008), pp. (157-165), ISSN 0300-8177

Staba, E.J.; Lash, L. & Staba, J.E. (2001). A commentary on the effects of garlic extraction and formulation on product composition. *The Journal of nutrition*, 131, 3s, (Mar 2001), pp. (1118S-1119S), ISSN 0022-3166

Sueyoshi, T.; Green, W.D.; Vinal, K.; Woodrum, T.S.; Moore, R. & Negishi, M. (2011). Garlic Extract Diallyl Sulfide (DAS) Activates Nuclear Receptor CAR to Induce the Sult1e1 Gene in Mouse Liver. *PloS one*, 6, 6, 2011), pp. (e21229), ISSN 1932-6203

Surh, Y.J. (2003). Cancer chemoprevention with dietary phytochemicals. *Nature reviews. Cancer*, 3, 10, (Oct 2003), pp. (768-780), ISSN 1474-175X

Takeyama, H.; Hoon, D.S.; Saxton, R.E.; Morton, D.L. & Irie, R.F. (1993). Growth inhibition and modulation of cell markers of melanoma by S-allyl cysteine. *Oncology*, 50, 1, 1993), pp. (63-69), ISSN 0030-2414

Takezaki, T.; Gao, C.M.; Ding, J.H.; Liu, T.K.; Li, M.S. & Tajima, K. (1999). Comparative study of lifestyles of residents in high and low risk areas for gastric cancer in Jiangsu Province, China; with special reference to allium vegetables. *Journal of epidemiology / Japan Epidemiological Association*, 9, 5, (Nov 1999), pp. (297-305), ISSN 0917-5040

Tanaka, S.; Haruma, K.; Kunihiro, M.; Nagata, S.; Kitadai, Y.; Manabe, N.; Sumii, M.; Yoshihara, M.; Kajiyama, G. & Chayama, K. (2004). Effects of aged garlic extract (AGE) on colorectal adenomas: a double-blinded study. *Hiroshima journal of medical sciences*, 53, 3-4, (Dec 2004), pp. (39-45), ISSN 0018-2052

Tanaka, S.; Haruma, K.; Yoshihara, M.; Kajiyama, G.; Kira, K.; Amagase, H. & Chayama, K. (2006). Aged garlic extract has potential suppressive effect on colorectal adenomas in humans. *The Journal of nutrition*, 136, 3 Suppl, (Mar 2006), pp. (821S-826S), ISSN 0022-3166

Tsai, C.W.; Yang, J.J.; Chen, H.W.; Sheen, L.Y. & Lii, C.K. (2005). Garlic organosulfur compounds upregulate the expression of the pi class of glutathione S-transferase in rat primary hepatocytes. *The Journal of nutrition*, 135, 11, (Nov 2005), pp. (2560-2565), ISSN 0022-3166

Tsai, C.W.; Chen, H.W.; Yang, J.J.; Sheen, L.Y. & Lii, C.K. (2007). Diallyl disulfide and diallyl trisulfide up-regulate the expression of the pi class of glutathione S-transferase via an AP-1-dependent pathway. *Journal of agricultural and food chemistry*, 55, 3, (Feb 7 2007), pp. (1019-1026), ISSN 0021-8561

Tsao, S.M. & Yin, M.C. (2001). In-vitro antimicrobial activity of four diallyl sulphides occurring naturally in garlic and Chinese leek oils. *Journal of medical microbiology*, 50, 7, (Jul 2001), pp. (646-649), ISSN 0022-2615

Upreti, M.; Chu, R.; Galitovskaya, E.; Smart, S.K. & Chambers, T.C. (2008). Key role for Bak activation and Bak-Bax interaction in the apoptotic response to vinblastine. *Molecular cancer therapeutics*, 7, 7, (Jul 2008), pp. (2224-2232), ISSN 1535-7163

Valko, M.; Rhodes, C.J.; Moncol, J.; Izakovic, M. & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-biological interactions*, 160, 1, (Mar 10 2006), pp. (1-40), ISSN 0009-2797

Velmurugan, B.; Mani, A. & Nagini, S. (2005). Combination of S-allylcysteine and lycopene induces apoptosis by modulating Bcl-2, Bax, Bim and caspases during experimental gastric carcinogenesis. *European journal of cancer prevention : the official journal of the*

European Cancer Prevention Organisation, 14, 4, (Aug 2005), pp. (387-393), ISSN 0959-8278

Verma S.K., J.V., Verma D. (2008). Garlic - "The spice of life": composition, cooking, chemistry and preparations. *Journal of Herbal Medicine and Toxicology*, 2, 2, 2008), pp. (21-28), ISSN 0973-4643

Vermeulen, K.; Van Bockstaele, D.R. & Berneman, Z.N. (2003). The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. *Cell proliferation*, 36, 3, (Jun 2003), pp. (131-149), ISSN 0960-7722

Viry, E.; Anwar, A.; Kirsch, G.; Jacob, C.; Diederich, M. & Bagrel, D. (2011). Antiproliferative effect of natural tetrasulfides in human breast cancer cells is mediated through the inhibition of the cell division cycle 25 phosphatases. *International journal of oncology*, 38, 4, (Apr 2011), pp. (1103-1111), ISSN 1019-6439

Wang, H.C.; Yang, J.H.; Hsieh, S.C. & Sheen, L.Y. (2010a). Allyl sulfides inhibit cell growth of skin cancer cells through induction of DNA damage mediated G2/M arrest and apoptosis. *Journal of agricultural and food chemistry*, 58, 11, (Jun 9 2010a), pp. (7096-7103), ISSN 0021-8561

Wang, Y.B.; Qin, J.; Zheng, X.Y.; Bai, Y.; Yang, K. & Xie, L.P. (2010b). Diallyl trisulfide induces Bcl-2 and caspase-3-dependent apoptosis via downregulation of Akt phosphorylation in human T24 bladder cancer cells. *Phytomedicine : international journal of phytotherapy and phytopharmacology*, 17, 5, (Apr 2010b), pp. (363-368), ISSN 0944-7113

Wargovich, M.J. (2006). Diallylsulfide and allylmethylsulfide are uniquely effective among organosulfur compounds in inhibiting CYP2E1 protein in animal models. *The Journal of nutrition*, 136, 3 Suppl, (Mar 2006), pp. (832S-834S), ISSN 0022-3166

Wei Z., L.B.H.S. (1998). Garlic inhibits free radical generation and augments antioxidant enzyme activity in vascular endothelial cells. *Nutrition research*, 18, 1998), pp. (61-70), ISSN

Weiner, L.; Shin, I.; Shimon, L.J.; Miron, T.; Wilchek, M.; Mirelman, D.; Frolow, F. & Rabinkov, A. (2009). Thiol-disulfide organization in alliin lyase (alliinase) from garlic (Allium sativum). *Protein science : a publication of the Protein Society*, 18, 1, (Jan 2009), pp. (196-205), ISSN 0961-8368

Weisberger, A.S. & Pensky, J. (1958). Tumor inhibition by a sulfhydryl-blocking agent related to an active principle of garlic (Allium sativum). *Cancer research*, 18, 11, (Dec 1958), pp. (1301-1308), ISSN 0008-5472

Welch, C.; Wuarin, L. & Sidell, N. (1992). Antiproliferative effect of the garlic compound S-allyl cysteine on human neuroblastoma cells in vitro. *Cancer letters*, 63, 3, (Apr 30 1992), pp. (211-219), ISSN 0304-3835

Wertheim, T. (1844). Untersuchung des Knoblauchöls. *Ann Chem Pharm*, 51, 1844), pp. (289-315), ISSN

Wu, C.C.; Sheen, L.Y.; Chen, H.W.; Kuo, W.W.; Tsai, S.J. & Lii, C.K. (2002). Differential effects of garlic oil and its three major organosulfur components on the hepatic detoxification system in rats. *Journal of agricultural and food chemistry*, 50, 2, (Jan 16 2002), pp. (378-383), ISSN 0021-8561

Wu, X.J.; Kassie, F. & Mersch-Sundermann, V. (2005). The role of reactive oxygen species (ROS) production on diallyl disulfide (DADS) induced apoptosis and cell cycle

arrest in human A549 lung carcinoma cells. *Mutation research*, 579, 1-2, (Nov 11 2005), pp. (115-124), ISSN 0027-5107

Wu, X.J.; Hu, Y.; Lamy, E. & Mersch-Sundermann, V. (2009). Apoptosis induction in human lung adenocarcinoma cells by oil-soluble allyl sulfides: triggers, pathways, and modulators. *Environmental and molecular mutagenesis*, 50, 3, (Apr 2009), pp. (266-275), ISSN 0893-6692

Xiao, D.; Pinto, J.T.; Soh, J.W.; Deguchi, A.; Gundersen, G.G.; Palazzo, A.F.; Yoon, J.T.; Shirin, H. & Weinstein, I.B. (2003). Induction of apoptosis by the garlic-derived compound S-allylmercaptocysteine (SAMC) is associated with microtubule depolymerization and c-Jun NH(2)-terminal kinase 1 activation. *Cancer research*, 63, 20, (Oct 15 2003), pp. (6825-6837), ISSN 0008-5472

Xiao, D.; Choi, S.; Johnson, D.E.; Vogel, V.G.; Johnson, C.S.; Trump, D.L.; Lee, Y.J. & Singh, S.V. (2004). Diallyl trisulfide-induced apoptosis in human prostate cancer cells involves c-Jun N-terminal kinase and extracellular-signal regulated kinase-mediated phosphorylation of Bcl-2. *Oncogene*, 23, 33, (Jul 22 2004), pp. (5594-5606), ISSN 0950-9232

Xiao, D.; Herman-Antosiewicz, A.; Antosiewicz, J.; Xiao, H.; Brisson, M.; Lazo, J.S. & Singh, S.V. (2005). Diallyl trisulfide-induced G(2)-M phase cell cycle arrest in human prostate cancer cells is caused by reactive oxygen species-dependent destruction and hyperphosphorylation of Cdc 25 C. *Oncogene*, 24, 41, (Sep 15 2005), pp. (6256-6268), ISSN 0950-9232

Xiao, D.; Lew, K.L.; Kim, Y.A.; Zeng, Y.; Hahm, E.R.; Dhir, R. & Singh, S.V. (2006a). Diallyl trisulfide suppresses growth of PC-3 human prostate cancer xenograft in vivo in association with Bax and Bak induction. *Clinical cancer research : an official journal of the American Association for Cancer Research*, 12, 22, (Nov 15 2006a), pp. (6836-6843), ISSN 1078-0432

Xiao, D.; Li, M.; Herman-Antosiewicz, A.; Antosiewicz, J.; Xiao, H.; Lew, K.L.; Zeng, Y.; Marynowski, S.W. & Singh, S.V. (2006b). Diallyl trisulfide inhibits angiogenic features of human umbilical vein endothelial cells by causing Akt inactivation and down-regulation of VEGF and VEGF-R2. *Nutrition and cancer*, 55, 1, 2006b), pp. (94-107), ISSN 0163-5581

Xiao, D. & Singh, S.V. (2006). Diallyl trisulfide, a constituent of processed garlic, inactivates Akt to trigger mitochondrial translocation of BAD and caspase-mediated apoptosis in human prostate cancer cells. *Carcinogenesis*, 27, 3, (Mar 2006), pp. (533-540), ISSN 0143-3334

Xiao, D.; Zeng, Y.; Hahm, E.R.; Kim, Y.A.; Ramalingam, S. & Singh, S.V. (2009a). Diallyl trisulfide selectively causes Bax- and Bak-mediated apoptosis in human lung cancer cells. *Environmental and molecular mutagenesis*, 50, 3, (Apr 2009a), pp. (201-212), ISSN 0893-6692

Xiao, D.; Zeng, Y. & Singh, S.V. (2009b). Diallyl trisulfide-induced apoptosis in human cancer cells is linked to checkpoint kinase 1-mediated mitotic arrest. *Molecular carcinogenesis*, 48, 11, (Nov 2009b), pp. (1018-1029), ISSN 0899-1987

Yamasaki, T. & Lau, B.H. (1997). [Garlic compounds protect vascular endothelial cells from oxidant injury]. *Nippon yakurigaku zasshi. Folia pharmacologica Japonica*, 110 Suppl 1, (Oct 1997), pp. (138P-141P), ISSN 0015-5691

Yang, E.; Zha, J.; Jockel, J.; Boise, L.H.; Thompson, C.B. & Korsmeyer, S.J. (1995). Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. *Cell*, 80, 2, (Jan 27 1995), pp. (285-291), ISSN 0092-8674

You, W.C.; Blot, W.J.; Chang, Y.S.; Ershow, A.; Yang, Z.T.; An, Q.; Henderson, B.E.; Fraumeni, J.F., Jr. & Wang, T.G. (1989). Allium vegetables and reduced risk of stomach cancer. *Journal of the National Cancer Institute*, 81, 2, (Jan 18 1989), pp. (162-164), ISSN 0027-8874

You, W.C.; Zhang, L.; Gail, M.H.; Ma, J.L.; Chang, Y.S.; Blot, W.J.; Li, J.Y.; Zhao, C.L.; Liu, W.D.; Li, H.Q.; Hu, Y.R.; Bravo, J.C.; Correa, P.; Xu, G.W. & Fraumeni, J.F., Jr. (1998). Helicobacter pylori infection, garlic intake and precancerous lesions in a Chinese population at low risk of gastric cancer. *International journal of epidemiology*, 27, 6, (Dec 1998), pp. (941-944), ISSN 0300-5771

Yuan, J.P.; Wang, G.H.; Ling, H.; Su, Q.; Yang, Y.H.; Song, Y.; Tang, R.J.; Liu, Y. & Huang, C. (2004). Diallyl disulfide-induced G2/M arrest of human gastric cancer MGC803 cells involves activation of p38 MAP kinase pathways. *World journal of gastroenterology : WJG*, 10, 18, (Sep 15 2004), pp. (2731-2734), ISSN 1007-9327

Zeng, T.; Zhang, C.L.; Song, F.Y.; Han, X.Y. & Xie, K.Q. (2009). The modulatory effects of garlic oil on hepatic cytochrome P450s in mice. *Human & experimental toxicology*, 28, 12, (Dec 2009), pp. (777-783), ISSN 0960-3271

Zhang, Y.W.; Wen, J.; Xiao, J.B.; Talbot, S.G.; Li, G.C. & Xu, M. (2006). Induction of apoptosis and transient increase of phosphorylated MAPKs by diallyl disulfide treatment in human nasopharyngeal carcinoma CNE2 cells. *Archives of pharmacal research*, 29, 12, (Dec 2006), pp. (1125-1131), ISSN 0253-6269

Zhang, Z.M.; Yang, X.Y.; Deng, S.H.; Xu, W. & Gao, H.Q. (2007). Anti-tumor effects of polybutylcyanoacrylate nanoparticles of diallyl trisulfide on orthotopic transplantation tumor model of hepatocellular carcinoma in BALB/c nude mice. *Chinese medical journal*, 120, 15, (Aug 5 2007), pp. (1336-1342), ISSN 0366-6999