Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 12, pp.24633-24641, December, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ROLE OF GARLIC DERIVED ALLYL SULFIDE IN INDUCING G2/M CHECKPOINT ARREST AND APOPTOSIS

***Shraddha Mondal**

Department of Biochemistry and Virology, NIRRH, Mumbai, India

ARTICLE INFO

Article History:
Received 25th September, 2015
Received in revised form
18th October, 2015
Accepted 07th November, 2015
Published online 30th December, 2015

Key words:
Allium sativum
Cyclin B1,
Cdc25, Cdk 1,
p53, Microtubule
Bcl-2, Bcl-xL,
XIAP, JNK, Akt

ABSTRACT

In the last two decades a huge repertoire of research on the anticancer activity of organo sulfur compounds derived from *Allium sativum* (Garlic) has provided critical insight about the potential of garlic as a potent anticancer agent. Allyl sulfur compounds from garlic not only has been shown to be a chemotherapeutic compound but also, based on a number of in-vitro, in-vivo and case controlled population based studies, that it has a chemo preventive activity and showing inverse relation with the intake of garlic and incidence of cancer. Garlic has been shown to have inhibitory effect in different types of cancer that include carcinoma, sarcoma, myeloma and leukemia, having same molecular targets in the respective phase of regulatory and cell demise events, a feature highly desirous of current and future anticancer drug. Garlic has basically been shown to arrest cell cycle at the G2/M check point and induce apoptosis by cell cycle dependent or independent manner. This review contains the brief highlight of the chemistry of the organo sulfur compounds (OSC) from garlic that have anticancer property and detailed elucidation of the function of G2/M cell cycle checkpoint regulating molecules (Cyclin B1, Cdc25, Cdk 1, p53, Microtubule), apoptosis regulators infesting multiple signaling pathways that include cell survival pathway (Bcl-2, Bcl-xL, XIAP), mitochondrial pathways, and protein kinase pathway (JNK, Akt) and how these event regulators are modulated by the allyl sulfur compounds of the garlic culminating in the cell cycle arrest and apoptosis of the immortal cells.

Citation*:* Shraddha Mondal, 2015. "Role of garlic derived Allyl sulfide in inducing g2/m checkpoint arrest and apoptosis", *International Journal of Current Research,* 7, (12), 24633-24641.

INTRODUCTION

Based on the origin and type of cells or tissue involved, Cancers are classified into four types. (a) Carcinoma: It arise from the epithelial cells that covers the outer and luminal surfaces like, skin, colon, etc. Approximately 90% of all human cancers are carcinomas; (b) Sarcoma: These include cancer of the connective tissues such as bone, muscle, cartilage, and fibrous tissue. Approximately 2% of all cancers are sarcomas; (c) Leukemia: they originate from blood cells, and (d) Lymphoma: they arise from cells of the immune system. Approximately, 8% of all cancers are leukemia and lymphomas.In terms of metastatics potentiality, there are two classifications of cancers; (a) Benign tumors or adenomas: when neoplastic growth remains clustered and focalized as a single mass; (b) Malignant tumor or adenocarcinoma: when tumor infiltrate normal tissue and spreads throughout the body (Hanahan and Weinberg, 2000). Cancer cells have been found to be distinct from normal cells in atleast six different ways.

****Corresponding author: Shraddha Mondal,***
Department of Biochemistry and Virology, NIRRH, Mumbai, India.

These characteristic are shared unanimously by cancer cells which include self dependency and sufficiency in growth signals, insensate to growth inhibitory signals, elusion of apoptosis, infinite replicative potential, sustained angiogenesis, and tissue irruption, and metastasis (Hanahan and Weinberg, 2000). The ability of tumor cells to grow is not only dependent on the increase in the rate of cell proliferation but also on the decrease in the rate of cell death. Therefore regulation of the cell cycle events at each cell cycle check point and apoptosis plays critical role in the development of cancer. Thus targeting cell cycle checkpoint events and apoptosis has been the choice of target for most of the currently used anticancer agents. Cancer is a leading cause of death worldwide, accounting for 7.6 million deaths (around 13% of all deaths) in 2008, a survey data published in World Health organization conducted by GLOBECON, ICRA. Lung, stomach, liver, colon and breast cancer cause the most cancer deaths each year. About 70% of all cancer deaths in 2008 occurred in low- and middle-income countries. Death from cancers worldwide are projected to continue rising, with an evaluated value of 13.1 million deaths in 2030 (World Health Organization, 2008).The development of cancers has been associated with dietary behavior. Prevalence of cancer differ in distribution among countries and the factors controlling such uneven distribution is not

completely clear but certain factors have been associated like epigenetic factors, environmental influence, lifestyle which involves diet, physical exercise. Diet is known to play a major role in the development of such disparity. The prevalence of certain type of cancers is in different parts of the world can be attributed in part to the dietary habit of the populations.

gene products involved in cell cycle checkpoints and cell death pathways as emphasized here by detailed elucidation of the role of the molecular targets in Cell cycle checkpoints and Apoptosis and how these gene products gets modulated (Table - 2).

Table 1. Modulation of Cell Cycle arrest and Apoptosis induced by Garlic derived Allyl Sulfides. Seven types of cancers have been shown over here and which OSC and more prominently allyl compounds kills different cancer cell lines of the respective group

Cell Types	Cell Lines	Garlic derived OSC	Reference
In-vitro			
Tumor cells			
Genitourinary cancer			
Prostate cancer	PC-3/DU145	DATS	22,37
Bladder	humanT24	DATS	48
Gastrointestinal cancer			
Liver	J5	DATS	15
Gastric			
Colorectal	SW80, Caco-2 and HT-29	DATS, DADS, SAMC	13, 32,38, 29
Brain Tumor			
Neuroblastoma	SH-SY5Y	DADS	44
Breast cancer	MCF7	DATS,	14, 35
Thoracic/H&N cancers			
Lungs	H358, H460	DATS	18
Melanoma	A375 and M14	DATS	41
Hematological cancer			
Leukemia			
Normal cells			
bronchialepithelial cell	BEAS-2B	DATS	18, 37
normal keratinocyte	HaCaT	DATS	47
In-vivo			
Prostate cancer (TRAMP)	PC-3	DATS	16
Xenograft	PC-3	DATS	39

One of the ancient dietary element being used in a number of physiological disparity is garlic, which has a very long history of being used as a potential natural agent in treating various ailments. Health benefits of *Allium* vegetables including garlic have been noted throughout recorded history, dating back to at least 1400BC (Richard and Rivlin, 2001).The preclinical in-vitro and animal studies along with population based case-controlled studies (Fleischauer *et al*., 2001) have shown chemo preventive and anticancer activity of garlic derived bioactive compounds in different types of cancers (Table - 1).

The most interesting outcome of such study is that garlic is a potent chemotherapeutic natural product alongside having a potential chemo preventive role. The bioactive component of garlic targets Cell cycle and Apoptosis by modulating the activities of various molecular regulators involved in cell cycle checkpoints and progression and cell death by Apoptosis (John, 2001; Anna Herman-Antosiewicz, ?: Organ sulfides, 2007). The anticarcinogenic properties of garlic in animals have been demonstrated by its inhibition of tumor initiation and tumor promotion. Garlic derived OSChas been shown to suppress multiple signaling pathways and inhibit cell proliferation, and activate cell death pathway of apoptosis. The chemo preventive action of garlic might be due to its ability to induce apoptosis by several pathways i.e, cell survival pathway (Bcl-2, Bcl-xL, XIAP), caspase activation pathway (caspase-8, 3, 9), mitochondrial pathways, and protein kinase pathway (JNK, Akt). Garlic directly or indirectly controls different gene or

BIOACTIVE COMPOUND

The chemistry of the *Allium stivum* is although very intricate but fairly understood and contains many sulfur-containing compounds. It displays a vast array of differences in its composition depending on the mode of extraction, temperature, time (Lynn *et al*., 2001). So, the chemical composition of the intact and processed garlic are different. The primary sulfur-containing compounds in intact garlic are g-glutamyl-S-allyl-L-cysteines and S-allyl-L-cysteine sulfoxides (alliin). Both are abundant as sulfur compounds in intact garlic. On average, a garlic bulb contains up to 0.9% γ-Glutamylcysteines and up to 1.8% Alliin. In addition to these cardinal sulfur compounds, intact garlic bulb also contain a small amount of SAC, but no allicin.γ-glutamylcysteines can be hydrolyzed and oxidized to form alliin, which accumulates during storage of intact garlic bulbs at cool temperatures. In processed garlic such as crushed, chewed or cutted, the vacuolar enzyme, alliinase, rapidly lyses the cytosolic S-allyl-L-cysteine sulfoxides (alliin) to form the cytotoxic and odoriferous alkyl alkane-thiosulfinates such as allicin. Alliin is the precursor of other sulfoxides which include allicin, methiin, (1)-S-(trans-1-propenyl)-L-cysteinesulfoxide, and cycloalliin. These sulfoxides, apart from cyloalliin, are converted into thiosulfinates (such as allicin) through enzymatic reactions when raw garlic is cut or crushed. Allicin and other thiosulfinates instantly decompose to other antitumerogenic bioactive compounds, such as diallyl sulfide (DAS),diallyl disulfide (DADS) and diallyl trisulfide (DATS), dithiins and ajoene.

Table 2. G2/M Cell cycle checkpoint and Apoptosis molecular regulators, signaling pathways that are modulated by Garlic derived Allyl Sulfides. It also shows all the other molecules that are modulated in response to the Allyl Sulfides

Processes and Molecular association with the inhibition	Regulation	Reference
Cell Cycle Inhibition		
Cyclin B1	i.Down-regulated	13
	ii.Up-regulated	14, 15
Cdk1	Down-regulated	18
Cdc25c	Down- regulated	18, 22
p53	Up-regulated	13
Microtubule	Destabilized	27,28,29
p21Cip1/Waf1	Up-regulated	32
ERK pathway		
Cdk7	Down-regulated	15
Chk-1		
Apoptosis		
Bad (Ser155 & Ser136 phosphorylation)	Decrease	37
Bax	Up-regulated	18,35,36,37,38,39
Bak	Up-regulated	18
Bcl-2	Down-regulated	36,40,41
Bcl-xL	Down-regulated	36,41
c-Jun N-Kinase(JNK)	Activate	29,44,
Akt	Inactivate	37,47
Apaf-1	Increase	38
XIAP	Decrease	39
PARP	Degradation	36,40
AP1	Increase	36
Mitochondrial Transmembrane Potential ($\Delta\Psi m$)	Decrease	29,38,48

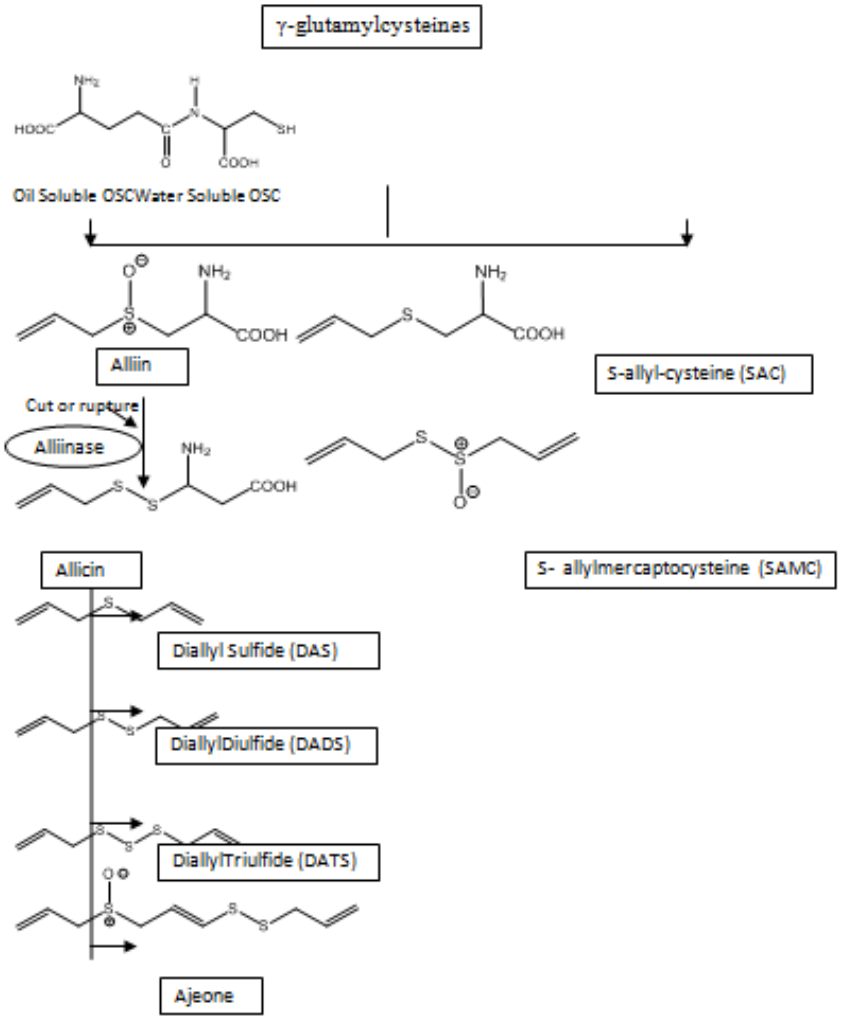

Figure 1. Description of garlic derived Organo Sulfur Compounds (OSC). The oil soluble sulfur compounds (DAS,DADS, DATS) and water soluble sulfur compounds (SAC, SAMC) are derive from the major chemical constituent of garlic, Allicin and γ-glutamylcysteines

Critical oil soluble components include diallyl sulfide (DAS), diallyl disulfide (DADS) diallyl trisulfide (DATS). Alcoholic or aqueous garlic extract primiraly contain g-glutamyl-S-allyl-L-cysteines, S-allyl-cysteine (SAC). Alternatively, the oil soluble ally compounds, can be slowly converted into water soluble allyl compounds, such as S-allyl-cysteine (SAC) and S-allylmercaptocysteine (SAMC). SAC, SAMC and trans-S-1-propenyl-L-cysteine (Trans SPC), together with a small amountof S-methyl-L-cysteine (SMC) are major constituents of Aged Garlic Extract (AGE). SAC is formed from γ-glutamyl cysteine catabolism and has been reported to contribute to the health benefits of some garlic preparations (Harunobu Amagase *et al*., 2001). A structural description of the allyl sulfides and other critical anticancer sulfur compounds derived from garlic have been shown in Figure 1.

MODE OF INHIBITION

The oil soluble, aqueous extracts and Aged Garlic Extract(AGE) have some specific organo-sulfur constituents that have been shown to have chemopreventive effect and anti-tumerogenic property in-vitro, in-vivo and small population based case control study. Garlic components induce cell cycle arrest by affecting diverse number cell cycle regulatory molecules that has been experimentally shown using pre-clinical in-vitro and in-vivo models. The modulatory effect of allyl sulfides on the G2/M phase check point have been presented in Figure 2 showing all the critically involved checkpoint molecules.

Cell Cycle Checkpoints

Cyclin B1

Cyclin B1, a G2-Phase cyclin, is a critical regulatory subunit that have imperative role in the transition at G2/M phase cell cycle checkpoint. Cyclin B1 bind to and activate a protein serine/threonine kinase subunit; cyclin B1 seems to bind exclusively to p34cdc2 (cdk1) (HarunobuAmagas, 2006). It mediates in transition to mitosis and its presence during chromosome condensation implicates another critical role in the G2/M cell cycle checkpoint. Binding of Cyclin B1 to its inactive partner Cdk1 induces conformational changes allowing Cdk1 to transform its phosphorylation status and to become an active kinase, the active cyclin B1-Cdk1 complex then, must translocate to the nucleus to begin phosphorylating nuclear substrates and initiate mitosis. Cyclin B1 moves into the nucleus just at the start of mitosis and associates with condensed chromosomes and the mitotic spindle (Pines *et al*., 1992). Phosphorylation of cyclin B1 within the CRS region enhances the import and inhibiting export of the cyclin B1-CDK1 complex from the nucleus thus citing another important regulatory role of cyclin B1 in the cyclin-Cdk complex at G2/M checkpoint to strictly regulate the cell cycle. Modulation in Cyclin B1 level has been implicated in the pathophysiology of different types of cancers (Porter *et al*., 2003).Garlic-derived organo-sulfur compounds (OSCs)15 including diallyl sulfide, diallyl disulfide (DADS) and/or diallyltrisulfide

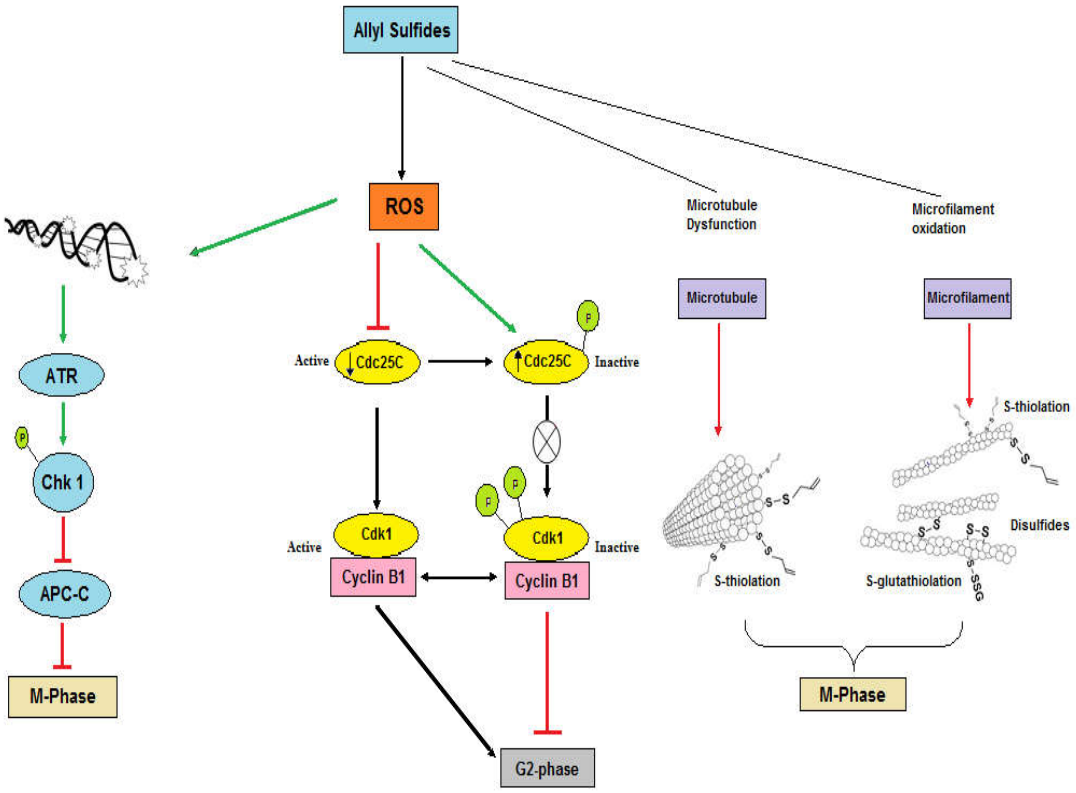

Figure 2 :Garlic derived allyl sulfides promotes G2/M phase cell cycle checkpoint arrest by modulation the activity of cell proliferation regulators. Ally sulfides mediated Reactive oxygen species (ROS) generation causes DNA damage resulting in the activation of ATR/ Chk1 dependent pro metaphase arrest. The activated ATR phosphorylate Chk 1 which block Anaphase Promoting Complex-Cyclosome (APC- C), thereby resulting in M phase arrest.The exact mechanism by which allyl sulfides causes activation of ATR remains elusive but may associate with the ROS dependent DNA double strand break. Allyl sulfide indiced G2 arrest may associate with the ROS dependent destruction of active Cdc25C and increase in inactive Ser 216 phosphorylated Cdc25C form which rather favors sequestration in cytosol by 14–3-3 protein, thus preventing the removal of inactivating tyr 15 phospho group from the inactive Cdk1/Cyclin B1 kinase complex, thereby inducing G2 arrest.Thiol oxidation via the formationof a mixed disulfide between Cys12and/or Cys354 of b-tubulin and themercapto-allyl group of allyl sulfides is infact culpable for mitotic spindledisassembly and Oxidation of actin microfilaments is another route for the M-phase arrest by the allyl compounds

(DATS) have shown to modulate the expression level of cyclin B1 in the cancers cells thereby inducing G2/M phase arrest. In human colon cancer SW480 cells, DADS was shown to down regulate cyclin B1(Viallard *et al*., 2001). But interestingly DATS in MCF-7 (Qian-Jin Liao *et al*., 2009) and DAS, DADS and DATS in liver cancer cell line J5increased the level of cyclin B1, which was correlated with cell cycle check points G2-M inhibition, judged by decrease in their percent in G2-M compared to control group. The higher level of cyclin B1 in the breast and liver cancer cell lines may give a possible mechanism of the sensitivity towards apoptosis in cells overexpressing cyclin B1 (Ahmed Malki *et al*., 2009).In-vivo study of Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) mice, for the first time, shows that DATS administration prevents progression to invasive carcinoma and lung metastasis in TRAMP mice with a significant induction in Cyclin B1 level in dorsolateral prostate (Wu *et al*., 2004).

Cell Dependent Kinase 1(cdk1 or cdc2)

Cell dependent Kinase 1, a group of serine/threonine kinase, is the sole kinase in association with a cyclin that is associated with the G2/M phase transition. Cdk1 kinase was the first kinase whose role in cell cycle transition was studies and was more prominently known as MPF (M-Phase Promoting Factor). cdc2 interacts primarily with cyclin B or homologous B type cyclins to regulate G2-M transition and the active form is activelyexcluded from the nucleus, where it must go to phosphorylate the substrates that will bring aboutthe various steps of mitosis (Shivendra *et al*., 2008). Cdc2 thus represents a critical regulatory molecule in the G2/M transition phase. Cdk therefore form an important component of cell cycle whose deregulation causes the deregulation of the core machinery that drives cell cycle progression and therefore to deregulation of the Cdk activity. The DATS-mediated G2-M phase cell cycle arrest was explained in H358 (anon-small cell lung cancer cell line) and H460 (a large cell lung cancer cell line) by down - regulation of cyclin-dependent kinase 1 (Cdk1) expression leading to accumulation of Tyr15 phosphorylated (inactive) Cdk1 (George *et al*., 2006).

CDC25

CDC25 phosphatases are not only rate-limiting activators of cyclin-dependent kinases (CDKs) but also important targets of the CHK1/CHK2-mediatedcheckpoint pathway. There are 3 isoform of the mammalian CDC25 family,i.e, CDC25A, CDC25B and CDC25C and each seems to exert unique biological functions. CDC25C is dispensable for bothmeiotic and mitotic divisions, although it is highly regulated during the reductional division and normal division processes (Shivendra, 2009). Activated CDC25C controls the activity of CyclinB1-Cdk1 complex at the G2/M phase by removing the inhibitory phosphate group from the cdk1 at Thr14 and Tyr15. CDC25 are regulated by 14-3-3 via phosphorylation during interphase (23–25) (Hiroaki Kiyokawa and Dipankar Ray, 2008). Deregulated expression of these phosphatases allows cells to overcome DNA damage-induced checkpoint, leading to genomic instability. The biological properties of CDC25c phosphatases provide significant insight into the pathobiology of cancer. (Hiroaki Kiyokawa and Dipankar Ray, 2008; Rose Boutros, 2007). DATS mediated Reactive Oxygen Species (ROS) formed by a high redox potential compound using the intracellular Glutathione (GSH) and DATS's homolytic cleavage product causes a decrease in the level of Cdc25C in human prostate cancer cell (Aressy and Ducommun, 2008). DATS was significantly more effective than either diallyl sulfide (DAS) or diallyl disulfide (DADS) against proliferation of human lung cancer cell lines H358 (anon-small cell lung cancer cell line) and H460 (a large cell lung cancer cell line) in decreasing the cell division cycle 25C proteinat the expression level (George *et al*., 2006).

P53

The transcription factor p53 has been reported to play a very important role in G2/M phase cell cycle arrest (10–12). As a tumor suppressor, p53 is responsible for protecting cells from tumerogenic alterations (227,228). P53 dependent cell cycle arrest has been observed in a number of cancer cell lines indicating the significance of p53 dependent cell cycle arrest due to DNA damage. The mechanism by which p53 regulates the G2/M transition involves regulation of the Cyclin dependent kinase (Cdc2) which is pivotal for entry into mitosis. DADS have been shown to down regulate p53 expression in human colon cancer cell lines resulting in cycle arrest at G2/M checkpoint (Viallard *et al*., 2001).

Microtubule

During mitosis, MT dynamics dramatically increase and the formation and tension of kinetochore MTs is important for the correct attachment with the centromere, separation of tetrads, and segregation of chromosomes (William *et al*., 2001). Indirect evidence suggests that the common mechanism underlying the inhibition of spindle MT dynamics, results in the deceleration of the metaphase-anaphase transition, defective segregation of chromosomes, mitotic block and subsequent induction of the mitochondrial pathway of apoptosis. (William *et al*., 2001; Eddy Pasquier1 and Maria Kavallaris, 2008) DATS basically mediates in thiol/Disulfide exchange by redox modification of specific reactive cysteines resulting in thiolyation of the protein like actin microfilament and β-tubulin causing depolymerization of actin filament and microtubule leading to M-Phase cell cycle arrest (Jordan and Wilson, 2004).

Hosono *et al*. demonstrated that treatments with DATS induced an increase in the molecular weight of Mt subunit, tubulin by 71.2 Da, which corresponds to the mass of the fragment of mercapto-allyl group originating from DATS, as well as from SAMC, indicating that S-allyl adducts to Cys12beta and Cys354beta are the main event in triggering microtubule network disassembly and inducing interphase arrest (Giuseppe Filomeni, 2008). It was reported that SAMC, but not S-allyl-cysteine (SAC), is able to interfere with microtubule polymerization by inducing thioloxidizing– dependent disruption of the microtubule network and therefore acts as a Microtubule Destabilizing agent. Formation of monopolar and multipolar spindles in mitotic human colon cancer cells SW480 and NIH3T3 mouse fibroblast cells (a well characterized model for studying Microtubule) which were in accordance with the

MT depolymerizing activity of SAMC (Hosono *et al*., 2005; Xiao *et al*., 2003). The role envisaged for tubulin sulphydryls in garlic derived allyl sulfide mediated cytotoxicity has put forward the question whether microfilaments could be also affected by similar course of action. On the basis of structural composition, the presence of numerous reactive cysteines residue on actin, it cannot be excluded that allyl compounds may induce redox modifications also at the actin level. Based on theinvolvement of cytoskeleton in NAD(P)H oxidase activation, as well as in cell adhesion and motility, it could have a critical role in the induction of cell cycle arrest and apoptosis by destabilizing the imperative cytoskeletal filaments of the cell.

P21 waf1/cip1

One of the key role of p21Cip1/Waf1 is the arrest in the G2/M transition. The function of the p21Cip1/Waf1 protein depends on its localization in the cell; it plays different roles in nucleus and in cytoplasm. The main role of p21Cip1/Waf1 in the nucleus is the cell cycle arrest in response to DNA damage (Danhua Xiao *et al*., 2003). p21Cip1/ Waf1 protein plays an important role in the transition of cells from G2 phase of the cellcycle into mitosis depending on the activated or inactivated form of Cdk1/CyclinB complex. To arrest the cell cycle in the G2/M checkpoint, Cdk1/CyclinBmust occur in inactive form. However, it was found that the activity of the Cdk1/CyclinB complex is regulated also by interactions with p21Cip1/Waf1 protein (Jana *et al*., 2011). Cdk1 can be inhibited by p21Cip1/Waf1 protein in three ways: Firstly, Ifp21Cip1/Waf1 protein is in the cell in high levels, it is able to bind directly to the Cdk/cyclin complex. Cdk1 is activated by phosphorylation at Thr161 by Cdk activating kinase (CAK). CAK is formed by Cdk7, CyclinH, and Mat1 (CDK-activating kinase assembly factor). Proteinp21Cip1/Waf1 interferes with the activating phosphorylationof CAK catalyzed by Cdk1. Secondly, The cell cycle arrest in G2 phase seem to be caused also by the interaction of p21Cip1/Waf1 protein with PCNA protein (Xiao *et al*., 2005). DADS significantly increased p21WAF1/CIP1 in both Caco-2 and HT-29 colon cancer cell lines. As p21waf1=cip1 protein is involved in the cell cycle progression and can induce cell cycle arrest in G1 or G2 phases, increased p21waf1=cip1 expression could explain the G2 cell cycle arrest induced by DADS in both Caco-2 and HT-29 cells (35) (Suxing Liu *et al*., 2003).

Apoptosis

Pro-Apoptotic Protein

In quiescent and healthy cells, the effectors are maintained in active state via complexation with repressors. Upon receiving cues, in the form of DNA damage and cellular stress, the activators(Bid,Bad) are stimulated and complete with effectors(Bax, Bak) for binding to the repressors(Bcl-2, Bcl-Xl)and, in doing so, not only do they neutralize the anti-apoptotic action of repressors but also unleash the pro-apoptogenicity of effectors. The effectors initiates apoptotic cell death by virtue of their ability to insert into the Mitochondrial Outermembrane (MOM) resulting the formation of mitochondrial pores. This provides a route for the liberation of apoptogenic factors such as cytochrome c into the cytosol and activates a class of aspartic acid protease called caspase, which in turn, demolish the cellular architecture by cleavage of proteins culminating in total cellular destruction (Nathalie Druesne1 *et al*., 2004; Jerry *et al*., 2008). The DATS-induced apoptosis in human lung cancer cell lines H358 (a non-small cell lung cancer cell line) and H460 (a large cell lung cancer cell line) correlatedwith induction of proapoptotic proteins Bax, Bak, and BID (George *et al*., 2006). In breast cancer cell lines MCF-7 and MCF-12a DATS-induced apoptosis was also correlated with the induction of pro-apoptotic Bax protein and p53 protein stabilization (Jerry *et al*., 2008). In Basal Cell Carcinoma (BCC), OSC were involved in mitochondrial apoptosis, including DATS-associated enhances in phospho-p53, proapoptotic Bax (Na *et al*., 2012). In prostate cancer cell lines PC-3 and DU145DATS treatment promoted mitochondrial translocation of BAD (Wang *et al*., 2012). DATS induces apoptotic cell death in human primary colorectal cancer cells through a mitochondria-dependant signaling pathway by increased reactive oxygen species (ROS) production in primary colorectal cancer cells. The mitochondria-dependant apoptotic signaling pathway was shown to be entangled, as determined by increase in the levels of cytochrome c, ,AIF,Apaf-1 and caspase-9 and caspase-3 in DATS-treated primary colorectal cancer cells. The reduction in the level of $\Delta\Psi m$ was associated with an increase in the Bax/Bcl-2 ratio which led to activation of caspase-9 and -3. (Dong Xiao and ShivendraV.Singh, 2006).PC-3 human prostate cancer xenograft in vivo study indicates that DATS administration inhibited growth of PC-3 xenografts in vivo in association with induction of Bax and Bak (Yu *et al*., 2012).

Anti-apoptotic Protein

The Bcl-2 family of proteins has come to be regarded as a central player in coupling apoptotic stimuli to determining the fate of cells to live or die. The Bcl-2 protein can be divided into three major groups: Activators such as Bid and Bad, Effectors such as Bax and Bak and Repressor such as Bcl-2, Bcl-xL and Bcl-w. BCL-2 family proteins (repressor) control mitochondrial outermembrane permeabilization (MOMP) by negatively regulating the activationof the pro-apoptotic BCL-2 effector molecules, BAX and BAK (Jerry *et al*., 2008). In Basal Cell Carcinoma (BCC), OSC were involved in mitochondrial apoptosis, including DATS-associated decreases in anti apoptotic Bcl-2 and Bcl-xl in BCC cell line (Na *et al*., 2012). Moreover, DATS induced both caspase-dependent and caspase-independent pathway of apoptosis. Caspase-dependent pathway was activated by the release of cytochrome c, apoptosis inducing factor, activation of Apaf-1, caspase-9, caspase 3 and PARP. Caspase-independent apoptotic pathway was activated by the release of caspase independent apoptotic proteins into the cytoplasm, such as HtrA2/Omi (apoptosis inducing factor)and endonuclease G, and further translocation of apoptotic-inducing factor to the nucleus and the activation of caspase cascade (Na *et al*., 2012; Dong Xiao *et al*., 2006). In human melanoma cell lines A375 and M14DATS inhibits growth of melanoma cellsin a time-dependent and dose-dependent manner by inducing apoptosis in association with down regulation of Bcl-2 and Bcl-xL (Wang *et al*., 2010).

Apoptotic Signaling pathways

The signal transduction pathways mediating Bcl-2 phosphorylation and their association with the regulation of apoptosis have been the subject of intense research.The modulation of apoptotic signaling pathways by naturally occurring garlic derived ally sulfides shows its another interesting mode of action to induce apoptosis via mitochondrial pathway. c-Jun NH2-terminal kinase (JNK)/c-Jun pathway is a direct activator of mitochondrial deathmachinery without other cellular components and providea molecular linkage from oxidative stress to themitochondrial apoptosis machinery.JNK might enableapoptosis by interfering directly with mitochondria, resulting in releaseof cytochrome c (Zhou *et al*., 2009) through destabilization of members of the Bcl-2 family (Hatai *et al*., 2000).Study of neuroblastoma cell SH-SY5Y with DADS, showed commitment to apoptosis through the activationof the mitochondrial pathway (Down-regulation of Bcl-2 , release of cytochrome c into the cytosol, and activation of caspase-9 and its downstream substrate caspase-3) and that apoptosis inductionwas highly dependent on the activation of the redox-sensitive c-Jun NH2-terminal kinase (JNK)/c-Jun pathway (Schroeter *et al*., 2003).. In another study on colon cancer cell line SW80, SAMC triggersJNK1 signaling pathwaysthat lead to apoptosis (Xiao *et al*., 2003).

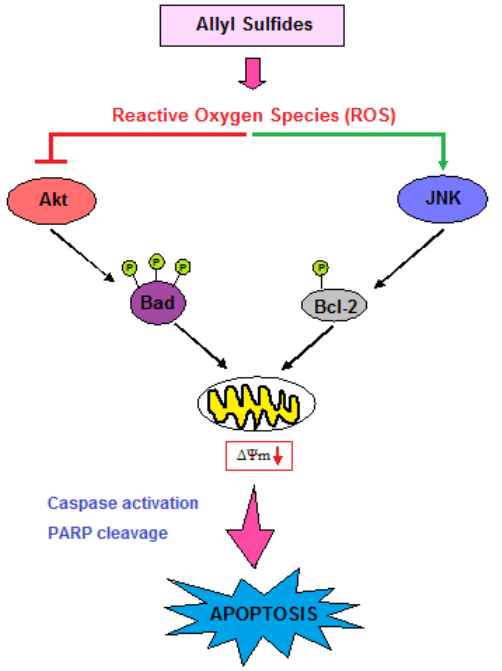

Figure 3. Garlic derived allyl sulfides (DADS, DATS) have been shown to modulate the protein Kinase pathways (c-Jun NH2-terminal kinase (JNK)/c-Jun pathway and Akt signaling pathways), probably by allyl sulfide mediated ROS generation and inactivation of Akt pathway and activation of JNK pathway. ROS sensitive Akt promote inactivating Ser^{155} and Ser^{136}phosphorylation on BAD (proapoptotic activator protein). ROS inactivates Akt thereby relieving BAD from inactivating phosphorylation from Akt. On the other hand, Allyl sulfides mediated induction of c-Jun NH2-terminal kinase (JNK)/c-Jun pathway activates JNK and resulting in the destabilization of anti-apoptotic repressor protein Bcl-2, thereby inducing apoptosis.

Akt promotes cell survival involves phosphorylation of pro-apoptotic Bcl-2 family member BAD. Activated Akt can phosphorylate (inactivating phosphorylation) several apoptosis regulating proteins including pro-apoptotic Bcl-2 family member BAD (Giuseppe Filomeni, 2003). BAD promotes cell death by interacting with anti-apoptotic members of Bcl-2 family such as Bcl-xL, which allows the multidomain (BH4-BH3-BH2-Bh1) pro-apoptotic Bcl-2 family members Bax and Bak to aggregate and cause release of apoptogenic molecules (e.g. cytochrome **c**) from mitochondria to the cytosol culminating into caspase activation and cell death (Datta *et al*., 1997). In a study with human prostate cancer cell line PC-3 and DU145 demonstrate that DATS inactivated Akt to trigger apoptosis in prostate cancer cells. DATS treatment (40 μM) caused a decrease in Ser^{155} and Ser^{136} phosphorylation of BAD (a proapoptotic protein), which is a downstream target of Akt. So, DATS treatment inactivates Akt to alleviate BAD phosphorylation, which leads to decreased interaction between BAD and 14-3-3β and mitochondrial translocation of BAD, thereby promoting apoptosis by BAD (Wang *et al*., 2012). In another in vitro studies on human T24 bladder cancer cells suggested that DATS could inhibit the growth of T24 cancer cell lines inadose- and time-dependent manner by inhibiting Akt signaling pathways and inducing apoptosis. The apoptotic response was associated with the up-regulation of Bax, down-regulation of Bcl-2 and caspase-3 activation (Wei *et al*., 2001).

Effects of Garlic on Normal Cells

Organo Sulfur Compounds derived from garlic like DADS and DATS have been shown to target tumor cells more specifically than the normal cells. Infact tumor cells were highly sensitive to OSC from garlic compared to normal cells, a feature highly desirable of a chemopreventive agent. Why such specific targeting occurs has not been completely understood but several reasons can be explained based on the observations from a huge repertoire of research on garlic and its action on mmortal cells. First, the role of Reactive Oxygen species (ROS) formed by ally compounds of garlic, acting by a redox mechanism within a cells causes the cancer cells to die by depleting GSH from the cancer cells, GSH maintains the redox state of the cells. Second, DATS kills tumor cell significantly more with minimal sensitivity towards normal cells. Akt Signaling pathway (Protein Kinase pathway) involved in cell proliferation, apoptosis is inhibited by DATS in prostate cancer cell line (PC-3, DU145), with significantly minimal inhibitory affect on normal cells. Likewise, DATS suppressed viability of cultured human lung cancer cell linesH358 (a non-small cell lung cancer cell line) and H460 (a large cell lung cancer cell line) by causingG2-M phase cell cycle arrest and apoptotic cell death with interestingly showing significantly more resistant to growth inhibition and apoptosisinduction by DATS compared with normal human bronchialepithelial cell line BEAS-2B. In BEAS-2B cell lines, DATS failed to induce BAD and BAK even at a concentration of 80uM, which is double the amount needed to kill a significant number of H358 and H460 lung cancer cell lines and PC-3 and DU145 human prostate cancer cell lines (George *et al*., 2006; Wang *et al*., 2012). In another experiment on skin cancer cell lines, melanoma A375 cells and basal cell carcinoma cells (BBC), DATS was found to be more effective in significantly killing cancer cells than normal keratinocyte HaCaT cells (Wei *et al*.,

2001; Hsiao-Chi Wang *et al*., 2010). So, the biochemical and signaling activity of the cell signaling pathways that control cell proliferation and apoptosis which gets unregulated in cancer cells compared to the normal cells could hypothetically be one of the reasons for the selective targeting of OSC to the cancer cells over normal cells.

Conclusion

However, a clear mechanism remained to be elucidated that how garlic derived allyl sulfide modulate cellular regulatory and cell signaling molecules that ultimately results in the imbalance of molecules favoring cell death and apoptosis of the immortal cells. Garlic derived Allyl sulfides targets a number of regulatory protein molecules and pathways that culminates in the G2/M phase checkpoint arrest and direct or indirect induction of apoptosis. Thus, highlighting a desirable trait that can influence or modulate as many target molecules as possible in arresting cell cycle at G2/M checkpoint or inducing apoptosis in different types of cancer cells. Moreover, from biochemical point of view, a distinct and clear mechanism needs to be resolved that is associated with the increase in phosphorylated product. That is, how allyl sulfides or allyl sulfides mediated ROS generation induces ROS generation Although, allyl sulfides like DAS, DADS, DATS, SAC, SAMC has been widely used to study anti-cancer potential, a systematic way must be used to characterize the critical constituents in an orderly manner that have the highest potentiality to kill cancer cells. In this review, DATS and DADS have been highly used along with some study from SAMC, SAC and few DAS study.

REFERENCES

Ahmed Malki, Muhammad El-Saadani and Ahmed S. Sultan, 2009. Garlic constituent diallyltrisulfide induced apoptosis in MCF7 human breast cancer cells, *Cancer Biology & Therapy* , November 15, 8:22, 2174-2184;

Anna Herman-Antosiewicz, Anna A Powolny, Shivendra V Singh, 2007 Sep. Molecular targets of cancer chemoprevention by garlic-derived Organosulfides, *ActaPharmacol Sin;* 28 (9): 1355–1364

Aressy, B. and Ducommun, B. 2008. Cell cycle control by the CDC25 phosphatases, *Anticancer Agents Med Chem.,* Dec;8(8):818-24.

Danhua Xiao, John T. Pinto, Jae-Won Soh, Atsuko Deguchi, Gregg G. Gundersen, Alexander F. Palazzo, Jung-Taek Yoon, HaimShirin, and I. Bernard Weinstein2, 2003. Induction of Apoptosis by the Garlic-Derived Compound S-Allylmercaptocysteine(SAMC) Is Associated with Microtubule Depolymerization and c-JunNH2-Terminal Kinase 1 Activation1, *Cancer Research,*63:6825-6837.

Datta, S.R., Dudek, H., Tao, X., Masters, S., Fu, H., Gotoh, Y. and Greenberg, M.E. 1997. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell*, 91, 231–241.

Dong Xiao and ShivendraV. Singh, 2006. Diallyltrisulfide, a constituent of processed garlic, inactivates Akt to triggermitochondrial translocation of BAD and caspase-mediated apoptosis in humanprostate cancer cells, Carcinogenesis vol.27 no.3 pp.533–540, doi:10.1093/ carcin/bgi228

Dong Xiao, Karen L. Lew, Young-Ae Kim, Yan Zeng, Eun-RyeongHahm, Rajiv Dhir, and Shivendra V. Singh, DiallylTrisulfide Suppresses Growth of PC-3 Human Prostate Cancer Xenograft In vivo in AssociationwithBax and Bak Induction, Clin Cancer Res 2006;12:6836-6843, DOI:10.1158/1078-0432.CCR-06-1273

Eddy Pasquier1 and Maria Kavallaris, 2008. Microtubules: A Dynamic Target in Cancer Therapy IUBMB Life, 60(3): 165–170.

Fleischauer, A.T. and Arab, L. 2001. Garlic and cancer: a critical review of the epidemiologic literature. *J Nutr.,* 131:S1032–40.

George, R., Stark and William R. 2006. Taylor, Control of the G2/M Transition, MolBiotechnol, ISSN: 1559-0305

Giuseppe Filomeni, Giuseppe Rotilio, and Maria R. Ciriolo, 2008. Molecular Transduction Mechanisms of the Redox Network Underlying the Antiproliferative Effects of Allyl Compounds from Garlic, *J. Nutr.,*

Giuseppe Filomeni, Katia Aquilano, Giuseppe Rotilio, *et al*,Reactive Oxygen Species-dependent c-Jun NH2-terminal, Kinase/c-Jun Signaling Cascade Mediates Neuroblastoma, 2003

Hanahan, D. Weinberg, R.A. 2000. The hallmarks of cancer. Cell. 100:57–70.

Harunobu Amagase, Brenda L. 2001. Petesch, Hiromichi Matsuura, Shigeo Kasugaand Yoichi Itakura, Intake of Garlic and Its Bioactive Components, J Nutr.,

HarunobuAmagase, Clarifying the Real Bioactive Constituents of Garlic, J Nutr, 2006

Hatai, T., Matsuzawa, A., Inoshita, S., Mochida, Y., Kuroda, T., Sakamaki, K., Kuida, K., Yonehara, S., Ichijo, H. and Takeda, K. 2000. Execution of apoptosis signal-regulating kinase 1 (ASK1)-induced apoptosis by the mitochondria-dependent caspase activation. J. Biol. Chem., *275:* 26576–26581.

Hiroaki Kiyokawa and Dipankar Ray, 2008. In vivo roles of CDC25 phosphatases: Biological insight into the anti-cancer therapeutic targets, Anticancer Agents Med Chem., December; 8(8): 832–836.

Hosono, T., Fukao, T., Ogihara, J., Ito, Y., Shiba, H., Seki, T. and Ariga, T. 2005. Diallyltrisulfide suppresses the proliferation and induces apoptosis of human colon cancer cells through oxidative modification of b-tubulin. *J. Biol. Chem.,* 280:41487–93

Hsiao-Chi Wang, Jen-Hung Yang,Shu-Chen Hsieh, And Lee-Yan Sheen, 2010. Allyl Sulfides Inhibit Cell Growth of Skin Cancer Cells throughInduction of DNA Damage Mediated G2/M Arrest and Apoptosis, *J. Agric. Food Chem.,* 58, 7096–7103, DOI:10.1021/jf100613x.

Jana C´ mielova´ and M. Rˇ eza´cˇova, Protein and its Function Based on Subcellular Localization, Journal of Cellular Biochemistry, 2011, 112:3502–3506 (2011)

Jerry, E. Chipuka, John C. Fisherb, Christopher P. Dillona, Richard W. Kriwackib,c, Tomomi Kuwanad,1, and Douglas R. 2008 . Greena Mechanism of apoptosis induction by inhibition of the anti-apoptotic BCL-2 proteins, PNAS _ December 23, _ vol. 105 _ no. 51 _ 20327–20332, doi_10.1073_pnas.0808036105

Jerry, E. Chipukand Douglas R. Green, 2008. How do BCL-2 proteins induce mitochondrial outer membrane permeabilization?, *Trends Cell Biol.,* April ; 18(4): 157–164. doi:10.1016/j.tcb.2008.01.007.

John, T. Pinto3 and Richard S. Rivlin, 2001. Antiproliferative Effects of Allium Derivatives from Garlic, *J. Nutr.,*

Jordan, M. A. and Wilson, L. 2004. Microtubules as a target for anticancer drugs. Nat. Rev. Cancer 4, 253–265.

Lynn, M. Knowles and John, A. 2001. Milner, Possible Mechanism by Which Allyl Sulfides Suppress Neoplastic Cell Proliferation, *J. Nutr.,*

Na, H.K., Kim, E.H., Choi, M.A., Park, J.M., Kim, D.H., Surh, Y.J. 2012. Diallyltrisulfide induces apoptosis in human breast cancer cells through ROS-mediated activation of JNK and AP-1, BiochemPharmacol. Nov 15;84(10):1241-50. doi: 10.1016/j.bcp.2012.08.024.

Nathalie Druesne1, Anthony Pagniez, Camille Mayeur, Muriel Thomas, Claire Cherbuy, Pierre-Henri Du_ee, Paule Martel and Catherine Chaumontet, 2004. Diallyl disulfide (DADS) increases histone acetylation and p21waf1=cip1 expression in human colon tumor cell lines, Carcin0genesis, vol.25 no.7 pp.1227--1236, 2004, DOI: 10.1093/carcin/bgh123

Pines, J., Hunter, T., Cyclins, 1992. A. and B1 in the human cell cycle.Ciba Found Symp.170:187-96; discussion 196-204.

Porter, L.A., Donoghue, D.J., Cyclin, B1 and CDK1, 2003. nuclear localization and upstream regulators, *Prog Cell Cycle Res.,* 5:335-47.

Qian-Jin Liao, Jian Su, Jie He, Ying Song, Hai-Lin Tang and Qi Su, 2009. Effect of diallyl disulfide on cell cycle arrest of human colon cancer SW480 cells, Chinese Journal of Cancer, February, 28:2, 138-141.

Richard, S. Rivlin, 2001. Historical Perspective on the Use of Garlic1,2, *J. Nutr.,*

Rose Boutros, ValérieLobjois & Bernard Ducommun, CDC25 phosphatases in cancer cells: key players? Good targets?, Nature Reviews Cancer 7, 495-507 (July 2007) | doi:10.1038/nrc2169

Schroeter, H., Boyd, C. S., Ahmed, R., Spencer, J. P., Duncan, R. F., Rice-Evans, C. and Cadenas, E. 2003. JNK-mediated modulation of brain mitochondria function: Ne target proteins for JNK signaling in mitochondria-dependent apoptosis. Biochem. J., published on-line: 10.1042 /BJ20030201.

Shivendra, V. Singh, 2009 April. Diallyl Trisulfide Selectively Causes Bax and Bak ediated Apoptosis in Human Lung Cancer Cells, Environ Mol Mutagen 50(3): 201–212. doi:10.1002/em.20431

Shivendra, V. Singh, Anna A. Powolny, Silvia D. Stan, Dong Xiao, Julie A. Arlotti, Renaud Warin, Eun-RyeongHahm, Stanley W. Marynowski, Ajay Bommareddy, Douglas M. Potter, and Rajiv Dhir, 2008 November 15. Garlic Constituent DiallylTrisulfide Prevents Development of Poorly-Differentiated Prostate Cancer and Pulmonary Metastasis Multiplicity in TRAMP Mice, *Cancer Res.,* 68(22): 9503–9511. doi:10.1158/0008-5472.CAN-08-1677

Suxing Liu , W. Robert Bishop, Ming Liu,Differential effects of cell cycle regulatory protein p21WAF1/Cip1 on apoptosis and sensitivity to cancer chemotherapy, Drug Resistance Updates 6 (2003) 183–195, doi:10.1016/S1368-7646(03)00044-X

Viallard, J.F., Lacombe, F., Belloc, F., Pellegrin, J.L., Reiffers, J. 2001. Molecular mechanisms controlling the cell cycle: fundamental aspects and implications for oncology, *Cancer Radiother,* Apr;5(2):109-29.

Wang, H.C., Hsieh, S.C., Yang, J.H., Lin, S.Y., Sheen, L.Y. 2012. Diallyltrisulfide induces apoptosis of human basal cell carcinoma cells via endoplasmic reticulum stress and the mitochondrial pathway, *Nutr Cancer.,* 64(5):770-80. doi: 10.1080/01635581.2012.676142.

Wang, H.C., Yang, J.H., Hsieh, S.C., Sheen, L.Y. 2010 Jun. Allyl sulfides inhibit cell growth of skin cancer cells through induction of DNA damage mediated G2/M arrest and apoptosis, J Agric Food Chem. 9;58(11):7096-103. doi: 10.1021/jf100613x.

Wei, M.C., Zong, W.X., Cheng, E.H., Lindsten, T., Panoutsakopoulou, V., Ross, A.J., Roth, K.A., MacGregor, G.R., Thompson, C.B. and Korsmeyer, S.J. 2001. Proapoptotic BAX and BAK: a r

William, R. Taylor, and George R. 2001. Stark, Regulation of the G2/M transition by p53, oncogene, 20, 1803 -1815

World Health Organization, Globocan 2008. IARC, 2013 (review edition)

Wu, C.C., Chung, J.G., Tsai, S.J., Yang, J.H., Sheen, L.Y. 2004. Differential effects of allyl sulfides from garlic essential oil on cell cycle regulation in human liver tumor cells, *Food ChemToxicol,* Dec;42(12):1937-47

Xiao, D., Herman-Antosiewicz, A., Antosiewicz, J., Xiao, H., Brisson, M., Lazo, J.S. and Singh, S.V. 2005. Diallyltrisulfide-induced G2-M phase cell cycle arrest in human prostate cancer cells is caused by reactive oxygen species dependent destruction and hyperphosphorylation of Cdc25C. Oncogene.24:6256–68.

Xiao, D., Pinto, J.T., Soh, J.W., Deguchi, A., Gundersen, G.G., Palazzo, A.F., Yoon, J.T., Shirin, H. and Weinstein, I.B. 2003. Induction of apoptosis by the garlicderived compound S-allylmercaptocysteine (SAMC) is associated with microtubule depolymerization and c-Jun NH2-terminal kinase activation. *Cancer Res.,* 63:6825–37.

Yu, C.S., Huang, A.C., Lai, K.C., Huang, Y.P., Lin, M.W., Yang, J.S., Chung, J.G. 2012. Diallyltrisulfide induces apoptosis in human primary colorectal cancer cells, Oncol Rep., Sep;28(3):949-54. doi: 10.3892/or.2012.1882.

Zhou, C., Mao, X.P., Guo, Q. and Zeng, F.Q. 2009. Diallyltrisulphide-induced apoptosis in human melanoma cells involves downregulation of Bcl-2 and Bcl-xL expression and activation of caspases, ClinExpDermatol. Dec;34(8):e537-43. doi: 10.1111/j.1365-2230.2009. 03594.x.

****