RESEARCH COMMUNICATION

Involvement of Multiple Signaling Pathways in Diallyl Sulfide Mediated Apoptosis in Mouse Skin Tumors

Neetu Kalra, Annu Arora, Yogeshwer Shukla*

Abstract

Many chemopreventive agents appear to target signaling intermediates in apoptosis-inducing pathways. Inherently, the process of neoplastic conversion selects against apoptosis to initiate, promote, and perpetuate the malignant phenotype. Thus, targeting apoptosis pathways in pre-malignant cells, in which these pathways are still relatively intact, may be an effective module of cancer prevention. Diallyl sulfide (DAS), a naturally occurring organosulfide, present in garlic, is reported to have pleiotropic biological effects. DAS is known to inhibit chemically induced tumors in a number of in vivo and in vitro studies. The aberration of tumor suppressor gene, p53 and the ras oncogene have been linked to the induction of multiple signaling pathways and to the resistance offered by cancer cells to the apoptosis. Therefore, the present study was carried out to investigate the role of DAS on modulation of multiple p53 and ras-induced signaling pathways in 7,12-dimethylbenathacene (DMBA) induced skin carcinogenesis. The results showed that DAS up regulates expression of tumor suppressor protein p53 (wt p53) and its downstream target molecule p21/waf1. Proapoptotic protein, bax was upregulated by DAS supplementation. An opposite trend was observed in DMBA induced antiapoptotic proteins expressions, survivin and bcl-2, which were significantly downregulated by DAS supplementation. In the present study we also demonstrated that DAS supplementation significantly reduces the expression of ras oncoprotein and to modulate expression of its signaling molecules including PI3K/Akt and MAPKs. Western blot analysis demonstrated that DAS significantly reduced the DMBA induced protein expressions of PI3K/Akt and p38MAPK. However, DAS supplementation did not alter the expression JNK1 and ERK1/2. Thus, our results confirm that DAS can adopt a multi-prong strategy to target multiple signaling pathways leading to induction of apoptosis and inhibition of growth of DMBA induced skin tumors in Swiss albino mice. Although studies of single pathways have been helpful in guiding investigations, new tools to study the integration and multiplicity of signaling pathways hold the hope of improved understanding of the signaling pathway alterations in cancer chemoprevention by naturally occurring compounds.

Key Words: Apoptosis - ras oncoprotein - wtp53 - DAS - multiple signaling pathways

Asian Pacific J Cancer Prev, **7**, 556-562

Introduction

Apoptosis, a physiological model of cell death, in which the cell itself executes the program for its own demise and subsequent removal, is an active field of research worldwide by scientists engaged in the search for cancer chemopreventive agents. Recent studies have identified several molecules common to the regulation of the cell cycle and apoptosis. Dual roles of these molecules such as ras, p53, survivin and bcl-2 provide a rational linkage between cell cycle and apoptosis (Vermeulen et al, 2003; Lin et al, 2005, Stoklsa and Golab 2005; Garcia et al, 2003). Among these factors, tumor suppressor p53 gene performs a pivotal role switching between cell cycle regulation and apoptosis induction (Vogelstein 2000). It is reported that differential transregulation by p53, which means transactivation or transrepression of different subsets of p53 target genes, was important to determine what downstream event would be elicited (Szak et al, 2001). Nonetheless, more details about the differential transregulation of p53 remains to be elucidated.

Central to the development of a large percentage of human cancers is the finding that the ras oncogene is frequently mutated to encode for abnormal ras proteins (i.e., oncogenic ras) that promote excessive cell proliferation (Cengel and McKenna, 2005; Illmer et al, 2005; Mammas et al, 2005). This mutation is considered an early genetic event in the development of various cancers and results in constitutive activation of an intracellular pathway leading to cellular proliferation. Several ras-induced signaling

*Environmental Carcinogenesis Division, Industrial Toxicology Research Centre, M.G.Marg; P.O.Box 80; Lucknow-226001 (India), *For Correspondence: Phone: (+91)094151-58430, Fax No. (+91) 522-2628227, Email: yogeshwer_shukla@hotmail.com, Shukla_y@rediffmail.com*

pathways such as the phosphatidylinositol 3-kinase (PI3K) /Akt (protein kinase B pathway) and mitogen activated protein kinases (MAPKs) pathways, have been linked to chemo-resistance of cancer cells (Carpentier 2005; Jiang et al 2005; Saleem et al, 2005). These findings suggest that both ras oncoprotein as well as p53 tumor suppressor protein could be important target for developing agents against cancer.

A number of studies demonstrated that evasion of apoptosis is one of the most important mechanisms of uncontrolled growth of tumor cells and resistance to the immune system. In the last decade, considerable attention has been focused on manipulation of apoptosis by natural products as a novel and promising strategy for cancer chemoprevention and therapy (Liu et al, 2005; Jiang et al, 2005; Karunagaran et al, 2005). A lot of such compounds exist in vegetables or fruits that are consumed by humans on a daily basis. Therefore, apoptosis induction by these agents in pre-cancerous and cancerous cells will undoubtedly contribute to chemoprevention. Clarification of the molecular mechanisms responsible for these effects may lead to the development of novel chemopreventive agents.

Among the many established naturally occurring dietary chemopreventive agents, the anticancer effects of sulfur-containing compounds (OSC) such as garlic constituents (GCs) and isothiocyanates (ITCs) have been widely reported. Since the 1980s, scientists have discovered that garlic possesses numerous medicinal properties. In particular, antimutagenesis and anticarcinogenesis effects of sulfur-containing compounds from garlic constituents received considerable attention. DAS, one of the OSCs present in garlic has shown to be effective both in vitro and in vivo against cancer in a variety of target organs such as skin, breast, lung, esophagus, colon and liver (Wu et al, 2001; Singh and Shukla, 1998a; 1998b; Pinto et al, 2001; Gerhauser et al, 2003; Boer et al, 2004; Gued et al, 2003; Singh et al, 2004). Recent studies showed that pleiotropic biological effects of DAS might involve the modulation of gene expression. DAS has been shown to enhance the expression of CYP1A1, 2B1 & 3A1 genes for drug metabolizing enzymes at the mRNA and protein levels (Wu et al, 2002). It has also been shown to possess inhibitory effects on the proliferation of tumor cells associated with induction of apoptosis (Hong et al, 2000, Kirlin et al, 1999). Earlier we reported that DAS could suppress DMBA induced skin tumors through induction of apoptosis (Arora et al, 2002). We further extended this work in the present study, taking DMBA induced mouse skin carcinogenesis model, we show that DAS is a potent multi-target anti-cancer agent and it induces apoptotic cell death of tumor cells via modulation of ras-induced PI3K/Akt, MAPKs and p53 mediated signaling pathways.

Materials and Methods

Materials

DMBA, DAS and antibody specific for b-actin (clone AC-74) were purchased from Sigma (St Louis, USA). The mouse monoclonal p38 MAPK survivin, phospho-Akt, PI3KB(85), ERK1/2, JNK1 antibody were procured from Cell Signaling Technology (Beverly, Massachusetts). Anti-p53 mouse monoclonal antibody specific for wild type protein (clone PAb 1620, ab-5), mouse monoclonal v-H-ras (clone F111-85, a-2), p21/waf 1 (ab-5) rabbit polyclonal IgG, bcl-2 (ab-2) rabbit polyclonal IgG and bax (ab-1) rabbit polyclonal IgG antibody were procured from Oncogene Research Products (Cambridge, USA). The rabbit anti mouse horseradish peroxidase or goat anti rabbit horseradish peroxidase conjugate secondary antibodies were obtained from Bangalore Genei (Bangalore, India). The nitrocellulose membranes were obtained from Sartorious, (Goettingen, Germany). The rest of the chemicals were of analytical grade of purity and were procured locally.

Animals and Treatment

Swiss albino mice (body weight 10-12 gm) were taken for the study from the Industrial Toxicology Research Centre animal-breeding colony. Animals were quarantined for one week on a 12/12 hr. light-dark cycle and were fed synthetic solid pellet diet (Ashirwad, India) and water ad libitum. The animals were divided into five groups comprising 25 animals each. The treatments were given topically on shaved dorsal skin as per dose and scheduled given below

Gr. I (Untreated) No Treatment
Gr.II (ACE+DMBA) 100ml Acetone applied topically followed 1 hr. later by 5mg DMBA/animal in 100ml acetone, 3 times/wk for 28 wks.
Gr.III (DAS+DMBA) 10-mg/kg b.wt. DAS applied topically in 100ml acetone followed 1 hr. later by 5mg DMBA/animal in 100m l acetone, 3 times/wk for 28 wks.
Gr.IV. (DMBA+DAS) 5mg DMBA/animal applied topically in 100ml acetone followed 1 hr. later by 10 mg/kg b.wt. DAS in 100m l acetone, 3 times/wk for 28 wks
Gr.V (ACE+DAS) 100ml Acetone applied topically followed 1 hr. later by 10-mg/kg b.wt. DAS in 100ml acetone, 3 times/wk for 28 wks

Animals from all the groups were examined every week for gross morphological changes including body weight changes and development of tumors locally on the skin during the entire study period. Skin from the painted area (with or without tumors) was excised and stored at –80˚C until use.

Preparation of Mouse Skin/Tumor Epidermal Lysates

The skin/tumor tissue was removed with sharp scalpel blades, and subcutaneous fat was scrapped off, on ice, epidermis from the whole skin was separated as described by Katiyar et al, (1999) and was homogenized in ice-cold lysis buffer (50mM Tris-Hcl, 150mM NaCl, 1mM EGTA, 1 mM EDTA, 20mM NaF, 100mM Na3VO4, 0.5% NP-40, 1% Triton X-100, 1mM PMSF (pH 7.4) with freshly added protease inhibitor cocktail (Protease Inhibitor Cocktail Set

III; Calbiochem, La Jolla, CA, USA). The homogenate was then centrifuged at 14000g for 25min at 4˚C and the supernatant (total cell lysate) was collected and aliquots were stored at –80˚C.

Western Blotting

Western blots were carried out as described by Towbin et al, (1979) in the mouse skin/tumor lysates mentioned above. Protein concentration was estimated by the routine method of Lowry et al, (1951) using BSA as a standard. Proteins (50 mg) were resolved on 10% SDS-polyacrylamide gels and electroblotted on nitrocellulose membranes. The blots were blocked overnight with 5% nonfat dry milk and probed with primary antibody at dilutions recommended by the suppliers. Immunoblots were detected by horseradish peroxidase conjugated anti mouse or anti rabbit IgG using chromagen 3,3'-diaminobenzidine tetrahydrochloride. To quantify equal loading, membranes were reprobed with b-actin antibody. The intensity of the bands was quantitated using Easy Win 32 software on Gel Documentation system (Herolab, GmbH, Wiesloch, Germany). Data were normalized to b-actin and expressed as mean values ±S.E. of three separate sets of experiments.

Results

Modulation of p53 mediated signaling pathway by DAS

Induction of p53 can result in apoptosis or growth arrest in cells with irreparable DNA damage (Levine, 1997). To validate the contribution of p53 in the apoptosis induced by DAS we first assessed the effect of DAS treatment on wild type p53 protein expression. Western blot analysis followed by densitometry analysis of the bands demonstrated that DAS supplementation resulted in significant increase in wt p53 protein expression (Figure 1) when given both prior (Gr. III) and post (Gr. IV) to DMBA treatment in comparison to untreated control Gr.I . It has been reported that wild type p53 upregulate the expression of its downstream regulator p21/waf1 (Murphy et al, 2002; Wang et al, 2003). To further address above findings we assessed the effect of DAS treatment on protein expression of p21/waf1 downstream, regulator of p53 gene. Western blot analysis followed by densitometry analysis showed significant increase in p21/waf1 (Figure 1B) expression by DAS supplementation in Gr III and Gr. IV in comparison to control Gr. I. DAS alone (Gr. V) had no effect on the levels of protein expression.

DAS induction of proapoptotic protein (bax) and downregulation of antiapoptotic proteins (survivin, bcl-2)

Survivin is a member of IAP family involving in both the apoptotic regulation and the cell-cycle control (Altieri 2003). It has been well documented that the expression of survivin is down regulated by p53 (Hoffman et al, 2002, Murphy et al., 2002, Yan 2005). Since our results showed that p53 level was activated by DAS treatment, it is possible that p53 can differentially regulate the survivin protein expression in our in vivo experimental model. To check this possibility we looked for the western blot analysis of survivin protein. Westernblot analysis demonstrated that DMBA treatment (Gr. II) resulted in significant upregulation of survivin (Figure 2) protein expression in comparison to control Gr. I. However DAS supplementation significantly reduced the DMBA induced protein expression of survivin in both Gr. III and Gr. IV. DAS alone (Gr. V) did not alter the expression survivin protein (Figure 2). p53 has been shown to regulate the expression of bcl-2 family genes (Wada et al, 2005; Wang et al, 2005). The ratio of proapoptotic bax and antiapoptotic bcl-2 is critically balanced during cell proliferation such that an increase in the level of bax or decrease in the level of bcl-2 can shift the ratio and trigger a signal initiating apoptosis (Antonsson et al, 1997; Katiyar et al, 2005). Our results showed that expression of proapoptotic protein bax was significantly increased by DAS supplementation in comparison to control Gr. I in both Gr. III and Gr. IV. Western blot analysis of bands revealed that DMBA caused significant increase in bcl-2 protein expression in comparison to control Gr. I. However significant decrease was observed in the DMBA induced (Gr. II) expression of bcl-2 protein by DAS treatment in both Gr. III and Gr. IV. DAS alone (Gr. V) did not cause any considerable change in proteins level. Thus DAS increased the bax/bcl2 ratio in favor of apoptosis.

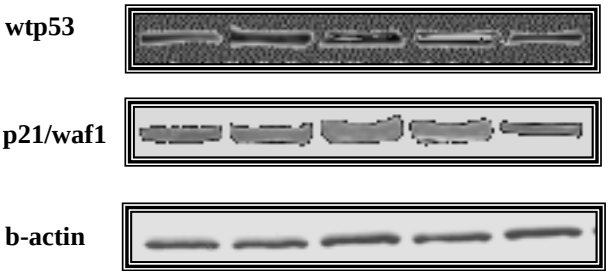

Figure 1. Effect of DAS Treatment on the Expression of wtp53 (A) and p21/waf1(B) Proteins in Skin/Tumor cells. Lane a. control, b. DMBA, c. DAS + DMBA, d. DMBA+ DAS, e. DAS alone.

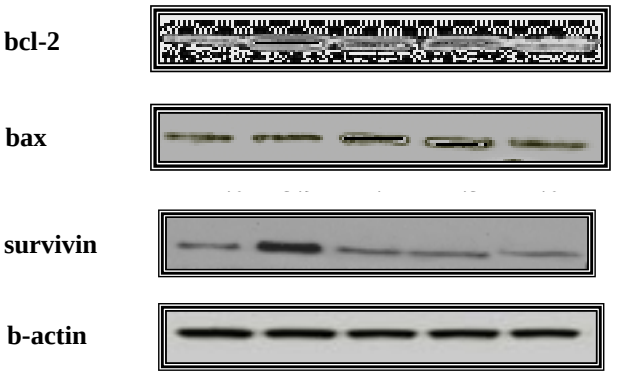

Figure 2. Effect of DAS Treatment on the Expression of survivin, bax and bcl-2 Proteins in Skin/Tumor Cells. Lane a. control, b. DMBA, c. DAS + DMBA, d. DMBA+ DAS, e. DAS alone.

DAS decreasedthe expression of ras oncoprotein and inhibited the PI3K/Akt signaling pathway (Figure 3). The frequent aberration in Ras signalling is reported to cause constitutive activation of PI3K/Akt during the development of cancer in humans. Thus we next investigated the effect of DAS treatment on expression of ras oncorotein. Western blot analysis of the bands showed that DMBA (Gr. II) caused significant increase in ras oncoprotein, PI3K (p85) proteins as well as phosphorylation of Akt protein levels in comparison to control Gr. I. However, DAS significantly downregulated the DMBA induced ras oncoprotein expression in both Gr. III and Gr. IV. Similarly DAS supplementation was found to cause a significant reduction in the DMBA induced expression of regulatory subunit of PI3K (p85) protein and significant decrease in the DMBA induced phosphorylation of Akt protein in both Gr. III and Gr. IV. However, the treatment of DAS alone (Gr. V) did not cause any change in the protein levels. These results suggest the inhibitory potential of DAS against PI3K/Akt pathway.

Modulation of MAPKs by DAS Treatment

Studies have linked the dysregulation of mitogen-activated protein kinases (MAPKs) with tumor cell survival in various cancer types. Therefore we determined whether MAPKs contributed to DAS-induced apoptosis in our experimental model We addressed this question by observing the effect of DAS treatment on expression levels of Erk1/2, JNK1 and p38 MAPK proteins. DMBA treatment (Gr. II) resulted in increased level of p38MAPK protein expression compared to control Gr. I while DAS supplementation resulted in significant decrease in the level of DMBA induced total p38 MAPK protein expression (Figure 4A) both in Gr. III and Gr. IV. However, DAS supplementation did not alter the level of JNK1 protein and Erk1/2 protein expression. Taken together, these observations indicate that activation of decrease in total expression level of p38 protein might be contributing towards apoptosis of tumor cells due to DAS.

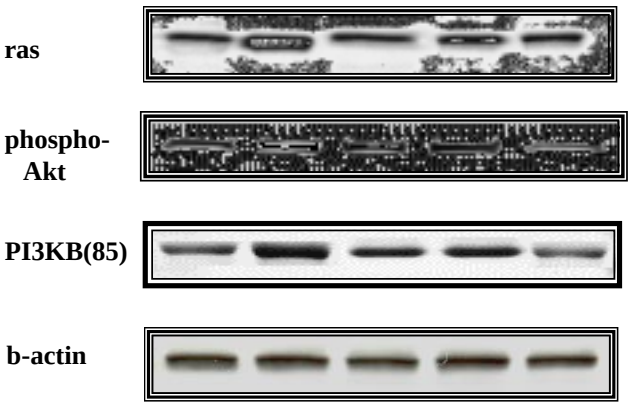

Figure 3. Effect of DAS Treatment on the Expression of ras (A), phospho-Akt (B) and PI3KB (85) (C) Proteins in Skin/Tumor Cells. Lane a. control, b. DMBA, c. DAS + DMBA, d. DMBA+ DAS, e. DAS alone.

Discussion

The balance between cell cycle procession and apoptosis is crucial for normal tissue homeostasis. Any breaks of this balance, which leads to inappropriate cell death and/or abnormal proliferation, can result in diseases even tumorigenesis (Giannetti et al, 2004; Sun et al, 2004). Apoptosis is arguably the most potent defense against cancer because it is the mechanism used by metazoans to eliminate deleterious cells (Sun et al, 2004). One promising approach could be identification of non-nutrient dietary constituents that can target multiple pathways without causing any undesirable toxicity. Here, we describe the usefulness of DAS, a novel non-nutrient dietary agent compound present in garlic.

Transformed cells have been shown to possess deregulated apoptotic machinery (Igney et al, 2002). Several pro-apoptotic proteins are either lost or diminished and many anti-apoptotic proteins are activated during the development of cancer (Igney et al, 2002). Other contributing molecular changes includes activation of oncogenes and inactivation of tumor suppressor genes. The tumor suppressor p53 protein plays a pivotal role in determining the quality of the response to DNA damage through its transcriptional activity. Upon DNA damage, p53 is activated by post-translational modifications, binds its cognate sequences on the promoters of its target genes and stimulates transcription (Papoutsaki et al, 2005). In our study we found that DAS upregulated the protein expression of wt p53 which in turn stimulate the transcription of p21/waf1. p53 mediated induction of p21/waf1 protein is an accordance to the earlier reports available in literature (Murphy et al, 2002; Wang et al, 2003). p21/waf1 appears to be important in p53-dependent growth arrest in cells that must undergo DNA repair (Waga et al, 1994) and its upregulation could be due to role of DAS in cell cycle arrest.

As is evident from our data, induction of both antiapoptotic proteins expression (survivin and bcl-2) by DMBA treatment during the course of tumorigenesis were found to be repressed by DAS supplementation. Induction of proapoptotic bax protein by DAS indicates that DAS may rectify the errors in apoptotic machinery of cancer cells. Earlier DAS was reported to induce wtp53 mediated apoptosis by modulating the bax/bcl2 ratio in non-small cell lung cancer cell lines (Hong et al, 2002). Another apoptotic protein, survivin taken in the present study, has been reported as a p53-regulated gene. Survivin is negatively regulated by wild-type p53 and participates in p53-dependent apoptotic pathway (Murphy et al, 2002, Hoffman et al, 2002). Thus increase in proapoptotic protein with concomitant decrease in apoptotic proteins expression can be well correlated with induction of wt p53 pathway by DAS supplementation.

Since DAS is a multi-target agent, other mechanisms of apoptosis could not be ruled out. Therefore we further studied the effect of DAS treatment on ras signaling pathway. The effects mediated by aberrantly activated ras in human cancers are likely to be very complex and are currently not

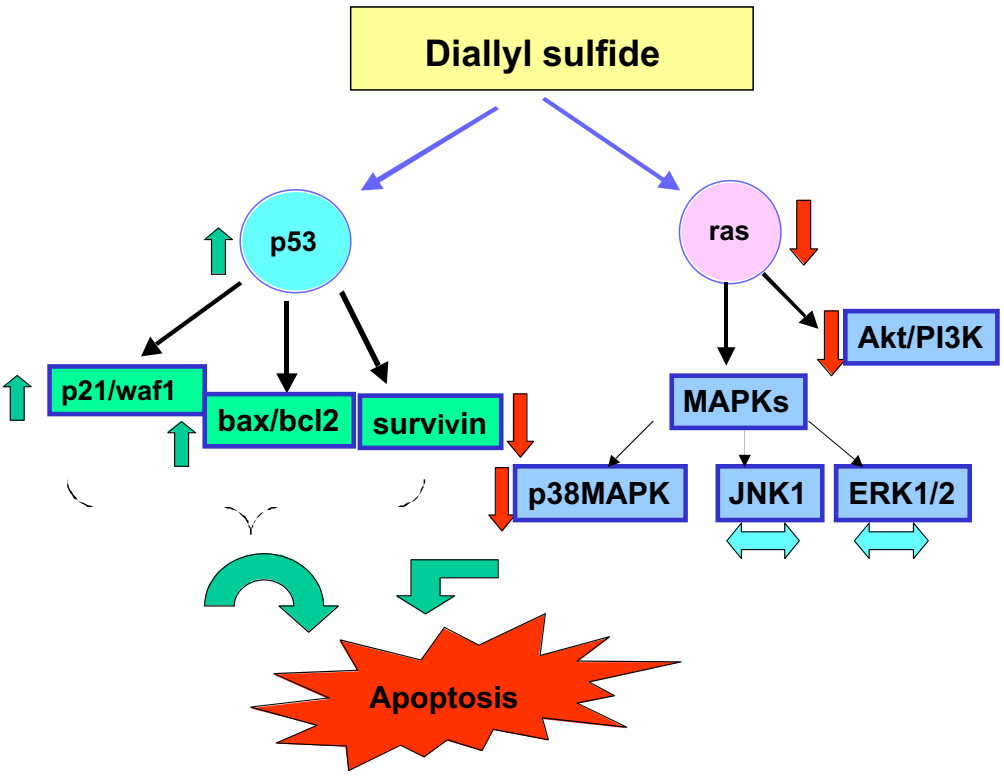

Figure 5. A Schematic Representation of the Action of DAS with Respect to Various Signaling Pathways Leading to Apoptosis in DMBA Induced Skin Tumors. Arrow upward represents increased expression; arrow downward represents decreased expression by DAS.

completely understood. The ras mutation results in the accumulation of ras oncoprotein and constitutive activation of various intracellular signaling pathways including PI3K/Akt and MAPKs, leading to cell proliferation (Sun et al, 2003, Asano et al, 2004). The growth-promoting potential of the PI3K/Akt pathway and its anti-apoptotic properties are closely linked to the resistance of cancer cells to a broad spectrum of apoptotic stimuli and several studies have demonstrated that treatment with the PI3K inhibitors substantially enhances apoptosis (Ng et al, 2000; Stoll et al, 2005; Poh and Parvez 2005, Mandal et al, 2005). Our findings clearly show that DAS inhibits overexpression of ras oncoprotein and ras induced PI3K/Akt signaling pathway. Recent reports are available in which ras signaling pathway has been targeted in induction of apoptosis (Saleem et al, 2005; Chang et al, 2003; Arase et al, 2000). Further both ras and PI3K/Akt signaling pathways have been reported to regulate the expression of MAPKs during the progression of various cancers (Kirschner 2002; Saleem et al, 2005, Xu et al, 2004). It has been demonstrated that p38 MAPK activation triggers an anti-apoptotic response to tumor cells, although there have also been some contrasting reports that p38 MAPK activation is central to the pro-apoptotic effects in some cancer cell lines (Grethe and Promeres , 2005, Engelbrecht et al, 2005). Recent reports have demonstrated a link between the ras oncoprotein and p38 MAPK activation in transformed cell (Shin et al, 2005, Saleem et al, 2005). It is evident from our data also that diallyl sulfide treatment significantly decreases the expression of p38 MAPK in mouse skin tumors leading to their apoptotic death. However DAS supplementation had no effect on the levels of JNK1 and ERK1/2 proteins expression. On the basis of the above findings we have proposed the model (Figure 5) showing involvement of multiple pathways in DAS induced apoptosis of mouse skin tumors. This study and the pathway that we have described herein is novel and have not been elucidated before. These pathways are interrelated and one cannot rule out role of these molecules in activating each other. Based on the outcome of this study, we suggest diallyl sulfide could provide a multi-prong beneficial strategy for targeting multiple signaling pathways leading to apoptosis. Identification of these mechanisms offers the possibility of designing novel targeted therapies for cancer in the future.

Acknowledgements

The authors are thankful to Dr.C.M.Gupta, Director, Industrial Toxicology Research Centre, Lucknow for his keen interest and support during the course of this study. Thanks are also due to Dr. Neeraj Mathur (Scientist) for statistically analyzing the data. Authors are thankful to Miss Jasmine George for helping in the preparation of this manuscript. The authors are also thankful to the Council of Scientific and Industrial Research (New Delhi, India) for providing a senior research fellowship to A.A.

References

Altieri DC (2003). Survivin, versatile modulation of cell division and apoptosis in cancer. *Oncogene*, **22**, 8581-9.

Antonsson B, Conti F, Ciavatta A, et al (1997). Inhibition of Bax channel-forming activity by Bcl-2. *Science*, **277**, 370–2.

Arase Y, Hiwasa T, Hasegawa R, et al (2000). Prevention of v-Ha-Ras-dependent apoptosis by PDGF coordinates in phosphorylation of ERK and Akt. *Biochem Biophys Res Commun*, **267**,33-9.

Arora A, Shukla Y (2002). Induction of apoptosis by diallyl sulfide in DMBA-induced mouse skin tumors. *Nutr Cancer*, **44**, 89-94.

Asano T, Yao Y, Zhu J, et al (2004). The PI 3-kinase/Akt signaling pathway is activated due to aberrant Pten expression and targets transcription factors NF-kB and c-Myc in pancreatic cancer cells. *Oncogene*, **23**, 8571-80.

Carpentier AF (2005). New therapeutic approaches in glioblastomas *Bull Cancer*, **92**, 355-9.

Cengel KA, McKenna WG (2005). Molecular targets for altering radiosensitivity: Lessons from Ras as a pre-clinical and clinical model. *Crit Rev Oncol Hematol*, **55**, 103-16.

Chang F, Steelman LS, Shelton JG, et al (2003). Regulation of cell cycle progression and apoptosis by the Ras/Raf/MEK/ERK pathway. *Int J Oncol*, **22**, 469-80.

de Boer JG, Yang H, Holcroft J, Skov K (2004). Chemoprotection against N-nitrosomethylbenzylamine-induced mutation in the rat esophagus. *Nutr Cancer*, **50**, 168-73.

Engelbrecht AM, Gebhardt S, Louw L (2006). Ex-vivo study of MAPK profiles correlated with parameters of apoptosis during cervical carcinogenesis. *Cancer Lett*, **235**, 93-9.

Garcia JF, Camacho FI, Morente M, et al (2002). Hodgkin and Reed-Sternberg Cells harbor alterations in the major tumor suppressor pathways and cell-cycle checkpoints: analyses using tissue microarrays. *Blood*, **101**, 681-9.

Gerhauser C, Klimo K, Heiss E, et al (2003). Mechanism-based in vitro screening of potential cancer chemopreventive agents. *Mutat Res*, **523-4**, 163-72.

Grethe S, Porn-Ares MI (2006). p38 MAPK regulates phosphorylation of Bad via PP2A-dependent suppression of the MEK1/2-ERK1/2 survival pathway in TNF-alpha induced endothelial apoptosis. *Cell Signal*, **18**, 531-40.

Gued LR, Thomas RD, Green M (2003). Diallyl sulfide inhibits diethylstilbestrol-induced lipid peroxidation in breast tissue of female ACI rats: implications in breast cancer prevention. *Oncol Rep*, **10**, 739-43.

Hoffman WH, Biade S, Zilfou JT, Chen J, Murphy M (2002). Transcriptional repression of the anti-apoptotic survivin gene by wild type p53. *J Biol Chem*, **277**, 3247-57.

Hong YS, Ham YA, Choi JH (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. *Exp Mol Med*, **32**, 127-34.

Igney FH, Krammer PH (2002). Death and anti-death: tumor resistance to apoptosis. *Nat Rev Cancer*, **2**, 277-88.

Illmer T, Thiede C, Fredersdorf A, et al (2005). Activation of the RAS pathway is predictive for a chemosensitive phenotype of acute myelogenous leukemia blasts. *Clin Cancer Res*, **11**, 3217-24.

Jiang H, Zhang L, Koubi D, et al (2005). Roles of Ras-Erk in apoptosis of PC12 cells induced by trophic factor withdrawal or oxidative stress. *J Mol Neurosci*, **25**,133-40.

Jiang H, Zhang L, Kuo J, et al (2005). Resveratrol-induced apoptotic death in human U251 glioma cells. *Mol Cancer Ther*, **4**, 554-61.

Karunagaran D, Rashmi R, Kumar TR (2005). Induction of apoptosis by curcumin and its implications for cancer therapy. *Curr Cancer Drug Targets*, **5**, 117-29.

Katiyar SK, Challa A, McCormick TS, Cooper KD, Mukhtar H (1999). Prevention of UVB-induced immunosuppression in mice by the green tea polyphenol (-)-epigallocatechin-3-gallate may be associated with alterations in IL-10 and IL-12 production *Carcinogenesis*, **20**, 2117-2124.

Katiyar SK, Roy AM, Baliga MS (2005). Silymarin induces apoptosis primarily through a p53-dependent pathway involving Bcl-2/Bax, cytochrome c release, and caspase activation. *Mol Cancer Ther*, **4**, 207-216.

Kirlin WG, Cai J, DeLong MJ, Patten EJ, Jones DP (1999). Dietary compounds that induce cancer preventive phase 2 enzymes activate apoptosis at comparable doses in HT29 colon carcinoma cells. *J Nutr*, **129**, 1827-35.

Kirschmeier P, Bishop WR, Nielsen LL, Pickett CB, Liu S (2002). Human survivin is negatively regulated by wild-type p53 and participates in p53-dependent apoptotic pathway. *Oncogene*, **21**, 2613-22.

Kirschner LS (2002). Signaling pathways in adrenocortical cancer. *Ann N Y Acad Sci*, **968**, 222-39.

Lens SM, Wolthuis RM, Klompmaker R, et al (2003). Survivin is required for a sustained spindle checkpoint arrest in response to lack of tension. *EMBO J*, **22**, 2934-47.

Levine AJ (1997). p53, the cellular gatekeeper for growth and division. *Cell*, **88**, 323-31.

Lin L, Ye Y, Zakeri Z (2005). p53, Apaf-1, caspase-3, and -9 are dispensable for Cdk5 activation during cell death. *Cell Death Differ*, **13**, 141-50.

Liu Y, Zhang YM, Song DF, Cui HB (2005). Effect of apoptosis in human breast cancer cells and its probable mechanisms by genistein. *Wei Sheng Yan Jiu*, **34**, 67-9.

Lowry OH, Rosenbrough NK, Farr AL (1951). Protein measurement with the Folin phenol reagent. *J Biol Chem*, **193**, 265-75.

Mammas IN, Zafiropoulos A, Spandidos DA (2005). Involvement of the ras genes in female genital tract cancer. *Int J Oncol*, **26**,1241-55.

Mandal M, Kim S, Younes MN, et al (2005). The Akt inhibitor KP372-1 suppresses Akt activity and cell proliferation and induces apoptosis in thyroid cancer cells. *Br J Cancer*, **92**,1899-905.

Murphy M, Mabruk MJ, Lenane P, et al (2002). Comparison of the expression of p53, p21, Bax and the induction of apoptosis between patients with basal cell carcinoma and normal controls in response to ultraviolet irradiation. *J Clin Pathol*, **55**, 829-33.

Ng SSW, Tsao MS, Chow S, Hedley DW (2000). Inhibition of p38 MAPK regulates phosphorylation of Bad via PP2A-dependent suppression of the MEK1/2-ERK1/2 survival pathway in TNF-alpha induced endothelial apoptosis. *Cancer Res*, **60**, 5451-5.

Papoutsaki M, Moretti F, Lanza M, et al (2005). A p38-dependent pathway regulates DeltaNp63 DNA binding to p53-dependent promoters in UV-induced apoptosis of keratinocytes. *Oncogene*, **24**, 6970-5.

Pinto JT, Rivlin RS (2001). Antiproliferative effects of allium derivatives from garlic. *J Nutr*, **131**,1058-60.

Poh TW, Pervaiz S (2005). LY294002 and LY303511 sensitize tumor cells to drug-induced apoptosis via intracellular hydrogen peroxide production independent of the phosphoinositide 3-

kinase-Akt Pathway. *Cancer Res*, **65**, 6264-74.
Saleem M, Kaur S, Kweon MH, et al (2005). Lupeol, a fruit and vegetable based triterpene, induces apoptotic death of human pancreatic adenocarcinoma cells via inhibition of Ras signaling pathway. *Carcinogenesis*, **26**, 1956-64.
Shin I, Kim S, Song H, Kim HR, Moon A (2005). H-ras-specific activation of Rac-MKK3/6-p38 pathway: Its critical role in invasion and migration of breast epithelial cells. *J Biol Chem*, **280**, 14675-83.
Singh A, Arora A, Shukla Y (2004). Modulation of altered hepatic foci induction by diallyl sulphide in Wistar rats. *Eur J Cancer Prev*, **13**, 263-9.
Singh A, Shukla Y (1998). Antitumour activity of diallyl sulfide on polycyclic aromatic hydrocarbon-induced mouse skin carcinogenesis. *Cancer Lett*, **131**, 209-14.
Singh A, Shukla Y (1998). Antitumor activity of diallyl sulfide in two-stage mouse skin model of carcinogenesis. *Biomed Environ Sci*, **11**, 258-63.
Stoklsa T, Golab J (2005). Prospects for p53-based cancer therapy. *Acta Biochim Pol*, **52**, 321-8.
Stoll V, Calleja V, Vassaux G, Downward J, Lemoine NR (2005). Dominant negative inhibitors of signalling through the phosphoinositol 3-kinase pathway for gene therapy of pancreatic cancer. *Gut*, **54**, 109-16.
Sun SY, Hail N Jr., Lotan R (2004). Apoptosis as a novel target for cancer chemoprevention. *J Natl Cancer Inst*, **96**, 662-72.
Szak ST, Mays D, Pietenpol JA (2001). Kinetics of p53 binding to promoter sites in vivo. *Mol Cell Biol*, **21**, 3375-86.
Towbin H, Staehelin T, Gordon J (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA*, **76**, 4350-4.
Vermeulen K, Berneman ZN, Van Bockstaele DR (2003). Cell cycle and apoptosis. *Cell Prolif*, **36**, 165-75.
Vogelstein B, Lane D, Levine AJ (2000). Surfing the p53 network. *Nature*, **408**, 307-10.
Wada T, Pippin JW, Marshall CB, Griffin SV, Shankland SJ (2005). Dexamethasone prevents podocyte apoptosis induced by puromycin aminonucleoside: Role of p53 and Bcl-2-Related family proteins. *J Am Soc Nephrol*, **16**, 2615-25.
Waga S, Hannon GJ, Beach D, Stillman B (1994). The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. *Nature*, **369**, 574-78.
Wang W, Rastinejad F, El-Deiry WS (2003). Restoring p53-dependent tumor suppression. *Cancer Biol Ther*, **2**, 55-63.
Wang XM, Chen JP, Fu WY, Xu LH (2005). Expression of P53 and Bax in rat liver induced by microcystin LR in vivo. *Zhejiang Da Xue Xue Bao Yi Xue Ban*, **34**, 220-2.
Wu CC, Sheen LY, Chen HW (2001). Effects of organosulfur compounds from garlic oil on the antioxidation system in rat liver and red blood cells. *Food Chem Toxicol*, **39**, 563-9.
Wu CC, Sheen LY, Chen HW (2002). Differential effects of garlic oil and its three major organosulfur components on the hepatic detoxification system in rats. *J Agric Food Chem*, **50**, 378-83.
Xu L, Pathak PS, Fukumura D (2004). Hypoxia-induced activation of p38 mitogen-activated protein kinase and phosphatidylinositol 3'-kinase signaling pathways contributes to expression of interleukin 8 in human ovarian carcinoma cells. *Clin Cancer Res*, **10**, 701-7.
Yan J, Yong W, Lei X, Ying Y, Jia Rui WU (2005). Differential regulation of survivin by p53 contributes to cell cycle dependent apoptosis. *Cell Res*, **15**, 361-70.