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Regulation of p21/ras protein expression by diallyl sulfide in DMBA induced neoplastic changes in mouse skin

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Abstract

Diallyl sulfide (DAS), a naturally occurring organosulfide, present in garlic, is known to possess pleiotropic biological effects. DAS is known to inhibit chemically induced tumors in a number of animal models. The chemopreventive properties of DAS seem to occur through a number of mechanisms, but its role on primary events on oncogenic activation is not well understood. In the present study, we demonstrated the modulatory effect of DAS on the expression of *H-ras* gene product, p21/ras protein as one of the mechanisms of its chemopreventive action in chemically induced mouse skin tumors. Our results showed that DAS administration leads to modulation of the DMBA-induced levels of p21/ras oncoprotein as early as 24 h after the DMBA application, suggesting down-regulation of the p21/ras by DAS. Furthermore, the modulatory effects of DAS were also evident in DMBA-induced mouse skin tumors. DAS administration led to increase in the levels of cytosolic p21/ras and decrease in the levels of p21/ras in membrane fractions. DAS administration was also found to down regulate the DMBA-induced *H-ras* mRNA level in mouse skin tumors. The immunohistochemical staining of the skin/tumor showed 55.82 and 46.86% decrease in the area positive for p21/ras expression levels in DAS pre- and post-supplemented groups, respectively. Flow-cytometric analysis, further confirms our results as indicated by a shift in the mean fluorescence intensity (MFI) towards lower fluorescence in DAS administered groups in comparison to the DMBA treated group. Thus, one mechanism of the growth inhibitory properties of DAS is through the suppression of development of tumors that harbor *ras* mutations by inhibiting the membrane association of oncogenic p21/ras protein.

Keywords: Organosulfur compound; Garlic; Diallyl sulfide; Oncogene; p21; H-ras signaling

1. Introduction

There is substantial epidemiologic data to suggest that healthy diet, that is, those high in fruits and vegetables, decrease the risk of a variety of cancers. For many dietary supplements, the precise biological mechanisms that underlie their chemopreventive activities are unknown. However, our understanding of the mechanisms underlying the anticancer activities for some dietary constituents is increasing. One example of such a constituent is diallyl sulfide (DAS). DAS is a lipid soluble organosulfur volatile compound from garlic, with characteristic pungent odor. DAS is a potent antioxidant with anti-inflammatory, antimutagenic and cancer preventive properties [1–4]. Epidemiological as well as laboratory studies have shown that DAS imparts chemopreventive effects against cancer in a variety of target organs such as skin, mammary, lung, colon and liver [2,3,5–7]. Recent studies have shown that

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pleiotropic biological effects of DAS might involve the modulation of gene expression. DAS supplementation is shown to enhance the expression of *CYP1A1*, *2B1* and *3A1* genes at the mRNA and protein levels [8]. These inhibitory effects are associated with induction of apoptosis and modulation of the expression of antiapoptotic (*bcl*−2), proapoptotic genes (*bax*) and the tumor suppressor gene *p*53 [9,10].

Parallel to the increased understanding of cancer chemopreventive potential of several dietary agents, there has been a marked expansion in understanding of the molecular mechanisms for carcinogenesis. 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. The *ras* family of proto-oncogene encodes 21 kD protein (p21/ras) which occupies a central position in the signal transduction pathway from the cell membrane to the nucleus and appears to play a critical role in the control of cell growth, differentiation and survival [11–13]. Oncogenic forms of *ras*, i.e. activation of normal *ras* genes by a single point mutation, have been described in about one half of almost all animal and human cancers in a wide variety of tissues [14,15]. The mutagenic effects involved in tumor initiation are of irreversible nature; therefore it is essential for the prevention of neoplasia to identify agents that are effective against the initially reversible propagation phase of tumor development. There are numbers of events during oncogenic activation, at which the function of the p21/ras can be inhibited, which may help in chemopreventive strategies [16]. Approaches to blocking *ras* function have included a number of varied methods such as blocking protein expression, post-translational processing, membrane anchorage, GTPase sensitivity, effecter interaction and selective targeting of cells in which *ras* signaling is active [16,17].

Since p21/ras protein plays a crucial role in the onset of tumorigenesis, attempts to achieve cancer chemoprevention during early stages of neoplastic changes at molecular level will be an effective approach. The review of literature showed only few studies targeted at p21/ras modulation by naturally occurring compound. In the present study we report the ability of DAS to modulate *ras*-linked tumorigenesis. The results confirm that DAS suppresses the growth of DMBA-induced mouse skin tumors, known to harbor *ras* mutations, by modulating the levels of ras protein.

2. Materials and methods

2.1. Materials

7,12-Dimethyl-benzanthracene, diallyl sulfide and antibody for β-actin (clone AC-74) were purchased from Sigma (St Louis, USA). The monoclonal v-H-ras (clone F111-85, Ab-2) antibody was procured from oncogene research products (Cambridge, USA). The horseradish peroxidase conjugate isotypes were obtained from Bangalore Genei (Bangalore, India) and the FITC conjugates were procured from Becton Dickinson (San Diego, CA, USA). The nitrocellulose membranes were obtained from Sartorious, (Gottingen, Germany). The rest of the chemicals were of analytical grade of purity and were procured locally.

2.2. p21/ras Studies

2.2.1. Short-term studies

The effect of DAS on the expression of p21/ras was investigated following DMBA exposure on mouse skin. Sixty male Swiss albino mice (body weight 10–12 gm) were taken from Industrial Toxicology Research Centre animal-breeding colony. The ethical approval for the experiment was obtained from institutional ethical committee. The animals were caged in polypropylene cages and housed six animals per cage on wood chip bedding in an air-conditioned (temperature 23 + 2 °C, relative humidity 55 + 5%) animal room. Animals were quarantined for one week on a 12/12 h light–dark cycle and were fed synthetic solid pellet diet (Ashirwad, Chandigarh, India) and water ad libitum. The animals were divided into five groups consisting of 12 animals each. The treatments were given topically on shaved dorsal skin as per dose and schedule given below

Gr. I-ACE	100 μl acetone applied topically, once only
Gr. II-ACE + DMBA	100 μl acetone applied topically followed 24 h later by 5 μg DMBA/animal in 100 μl acetone
Gr. III-DAS + DMBA	10 mg/kg b.wt. DAS applied topically in 100 μl acetone followed 24 h later by 5 μg DMBA/animal in 100 μl acetone
Gr. IV-DMBA + DAS	5 μg DMBA/animal applied topically in 100 μl acetone followed 24 h later by 10 mg/kg b.wt. DAS in 100 μl acetone
Gr. V-ACE + DAS	100 μl acetone applied topically followed 24 h later by 10 mg/kg b.wt. DAS in 100 μl acetone

Four animals from each group were euthanized by cervical dislocation after 24, 48 and 72 h of last treatment, respectively. The skin from the painted area was excised out, washed in chilled PBS and fat layer was scraped off with