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Mutation Research 589 (2005) 81–102

Reviews in Mutation Research

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Review

Induction of apoptosis in tumor cells by naturally occurring sulfur-containing compounds

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Received 27 July 2004; received in revised form 3 November 2004; accepted 3 November 2004
Available online 4 January 2005

Abstract

Chemoprevention is regarded as one of the most promising and realistic approaches in the prevention of human cancer. Among naturally occurring products, sulfur-containing compounds (OSCs), especially garlic compounds (GCs) and isothiocyanates (ITCs), represent two important and promising chemopreventive families because of their potent chemopreventive effects in various in vivo and in vitro models. In recent years, numerous investigations have shown that sulfur-containing compounds induce apoptosis in multiple cell lines and experimental animals. In the course of apoptosis induction by GCs and ITCs, multiple signal-transduction pathways and apoptosis intermediates are modulated. In particular, modulation of MAPKs and production of reactive oxygen species (ROS) seem to play pivotal roles in apoptosis induction by most GCs and ITCs. However, the role of P53 is still controversial. Based on present knowledge, GCs and ITCs may target not only the metabolism of carcinogens but also apoptosis signaling molecules. The effects of ITCs and GCs at multiple points of cancer development make these compounds highly promising candidates in cancer chemoprevention. However, the mechanisms of their anticancer effects are not fully understood, and further studies are required, especially to elucidate the role of cell-death receptors (the extrinsic pathway) and whether these agents induce apoptotic effects in non-tumor cells.
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Keywords: Apoptosis; Sulfur containing compound; Garlic constituents; Isothiocyanates

Abbreviations: AITC, allyl-isothiocyanate; ARE, antioxidant response element; BITC, benzyl-isothiocyanate; DADS, diallyl disulfide; DAS, diallyl sulfide; DATS, diallyl trisulfide; HSPs, heat shock proteins; JNK, c-Jun N-terminal kinases; MAPKs, the mitogen-activated protein kinases; NAC, *N*-acetylcysteine; NAG-1, nonsteroidal anti-inflammatory drug (NSAID)-activated gene; NF-κB, nuclear factor kappa B; OSCs, organosulfur compounds; PARP, poly(ADP-ribose) polymerase; PBITC, phenylbutyl isothiocyanate; PEITC, phenylethyl isothiocyanate; PHITC, phenylhexyl isothiocyanate; PITC, phenyl isothiocyanate; PMITC, phenylmethyl isothiocyanate; PPITC, phenylpropyl isothiocyanate, 4-phenylbutyl isothiocyanate; ROS, reactive oxygen species; SAC, *S*-allylcysteine; SFN, sulforaphane; SMAC, *S*-allylmercaptocysteine

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doi:10.1016/j.mrrev.2004.11.001

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1. Introduction

In the last decades, mounting evidence from mechanistic studies of cancer helped to develop new promising chemopreventive agents. On the basis of the mechanism through which they exert anticancer effects, chemopreventive agents can be divided into two groups: antimutagenic and antiproliferative [1]. Antimutagens reduce formation of mutagens or carcinogens thereby preventing DNA damage. For instance, ROS scavenging and alteration in carcinogen metabolism (through suppression of phase I enzymes or enhancement of phase II detoxifying enzymes) represent antimutagenic effects. Alternatively, chemopreventive agents may exert antiproliferative effects via induction of cell cycle arrest or apoptosis, inhibition of angiogenesis, induction of terminal differentiation, and inhibition of oncogene activity or DNA synthesis [2]. Recent chemoprevention strategies are more concerned with identifying substances with antiproliferative or antiprogressive activities [3]. In particular, 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. Numerous 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. Hence, apoptosis of initiated and/or neoplastic cells represents a protective mechanism against neoplastic transformation and development of tumors through elimination of genetically damaged cells or cells that may have been inappropriately induced to divide by mitogenic and proliferative stimuli [4]. In the last decade, considerable attention has been focused on manipulation of apoptosis as a novel and promising strategy for cancer chemoprevention and therapy [5–9]. To achieve this goal, different naturally occurring compounds such as resveratrol, curcumin and genistein have been studied and were found to induce apoptosis in malignant cells [10–12]. 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. Besides their well-known effect in modulating phase I and II enzymes, GCs and ITCs

induced apoptosis in vitro and in vivo. The aim of this paper is to give an overview on the progress made thus far in the study of apoptosis induction by GCs and ITCs and the signal transduction pathways involved.

2. Apoptosis induction by garlic constituents

Garlic, *Allium sativum*, is a member of the lily family that has been cultivated by humans as a food plant for over 10,000 years. Egyptian records dating to about 1550 B.C. mention garlic as a remedy for a variety of diseases [13]. 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. Experimentally, garlic and its associated sulphur components are reported to suppress the incidence of tumors of rodent models in breast, colon, skin, uterus, oesophagus and lung [14–20]. Epidemiological findings also demonstrated an inverse relationship between garlic consumption and the incidence of stomach cancer, colorectal cancer and prostate cancer [21–25]. Two pathways are involved in the conversion of natural garlic to sulfur compounds. The first pathway is natural aging bioconversion, which leads to the formation of mainly water soluble sulfur compounds such as *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SMAC) (chemical structures and bioconversion pathways are shown in Figs. 1 and 2). The second pathway is cell decomposition to allicin, which again breaks down rapidly under uncontrollable chemical reactions to produce odorous oil-soluble sulfur compounds, namely diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS) and ajoene (chemical structures and bioconversion pathways are shown in Figs. 1 and 2).

Chemopreventive effects of garlic constituents are based on the following mechanisms: (i) enhancement of the activity of specific mixed-function oxidases that

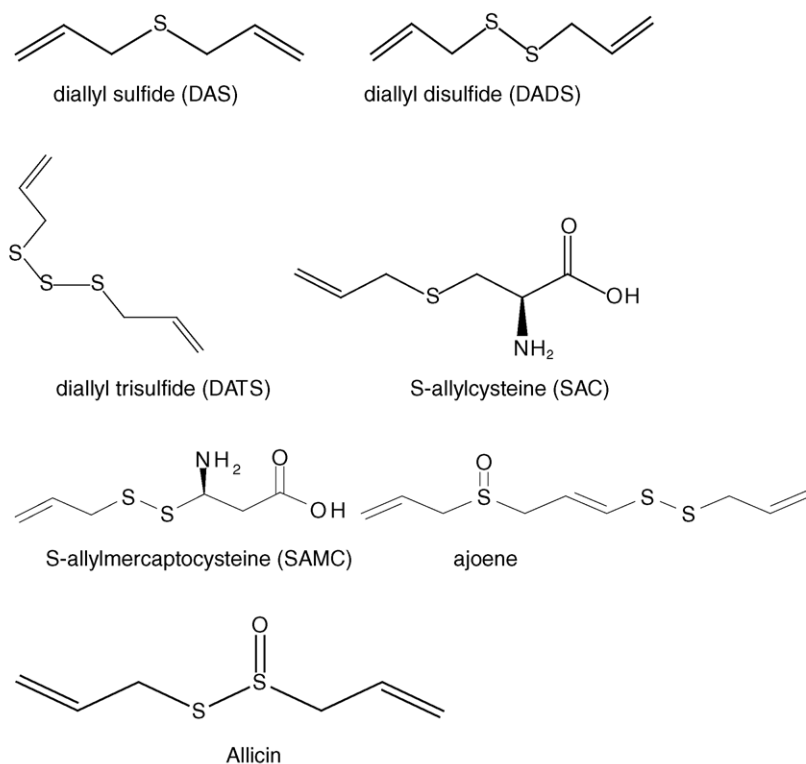

Fig. 1. Chemical structures of GCs.

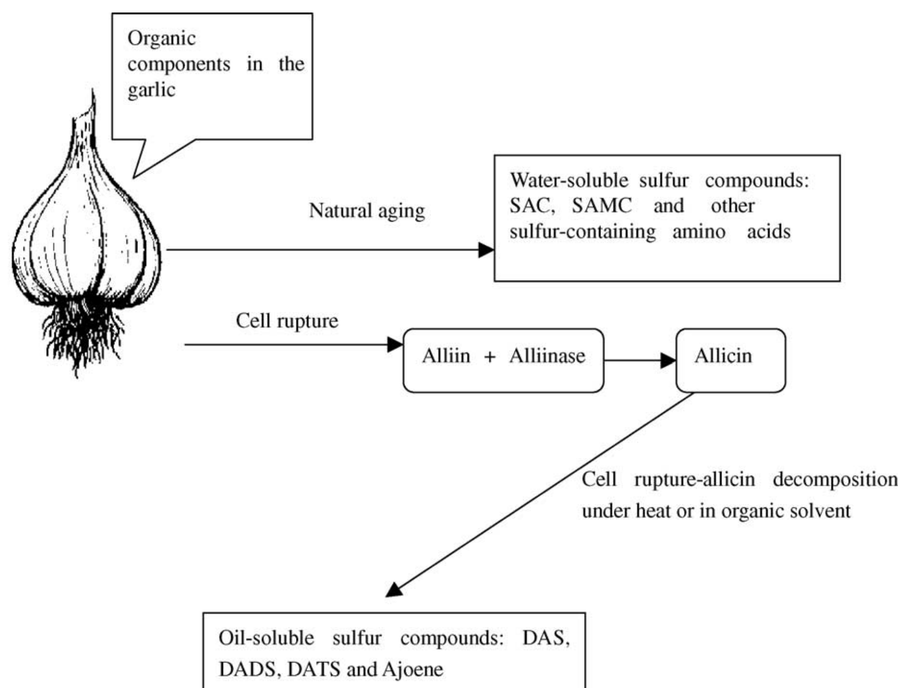

Fig. 2. Bioconversion pathway of GCs.

depress the activation of carcinogens [26–30], (ii) induction of phase II enzymes which enhance detoxification and excretion of potential carcinogens and reduction of the formation of DNA adducts [31–34], (iii) increased synthesis of GSH, an endogenous tripeptide thiol that directly protects cells from damage by free radicals [35], and (iv) induction of apoptosis (summarized in Table 1).

Apoptosis induction by DADS, DAS, SAC, SAMC, DAPS, allicin, and ajoene, all found in garlic, have been investigated in human colon, lung, prostate, leukemic, gastric, basal cell and mammary cancer cells in vitro (summarized in Table 1). There are also two in vivo studies in which SAC and DAS, respectively, induced apoptosis in male Syrian hamster buccal pouch (HBP) carcinogenesis model [36] and mouse skin tumors [37]. Generally, induction of apoptosis by GCs was dose and time-dependent. However, the kinetics of sub-diploid DNA content or caspase-3 activation were cell-model dependent. Previous studies showed that oil-soluble GCs, DAS, DADS and DATS were more effective in inhibiting the growth of canine mammary tumor cells than water-soluble GCs, *S*-allyl-cysteine, *S*-ethyl-cysteine and *S*-propyl-cysteine [38]. Also, in human colon cancer cells, oil-soluble DADS demonstrated stronger apoptotic potential than isomolar quantities of the water-soluble compound, *S*-allyl cysteine [39]. Perhaps, this dichotomous effect between oil-soluble and water-soluble GCs is attributed to their property of lipotropy, which may affect their efficiency of passing cell membrane. Interestingly, SAMC, a water-soluble GC with two sulfur atoms induced more significant apoptosis than its one sulfur atom counterpart, SAC, in SW-480 and HT-29 cells [40]. Similar effects were observed in cell proliferation studies [38]. Also, DADS, an oil soluble GC with two sulfur atoms was found to be more effective than one sulfur atom GC, DAS, in inducing apoptosis of non small cell lung cancer cells [41]. The mechanism of this sulfur atom-dependent effect is still unknown but the results from the aforementioned studies imply that higher number of sulfur atoms in GCs is responsible for a stronger biological activity. Unlike polysulfide compounds such as DADS and SAMC, DAS and SAC do not exhibit the same type of reactivity with cysteine and GSH [42]. Therefore, it is reasonable to speculate

Table 1
Apoptosis induction by GCs

GCs	Cell lines/animal model	Mechanisms/remarks[a]	Reference
DAS	HT29 cells, human colon caner	Negative	[149]
	Mouse, DMBA-induced skin tumors	Mechanism was not studied	[37]
	H460 cells, p53-wild type human lung cancer	Up-regulated Bax and down-regulated Bcl-2	[41]
	H1299 cells, p53-null human lung cancer	Mechanism was not studied	[41]
DADS	SH-SY5Y cells, human neuroblastoma	ROS and JNK dependent	[55]
	HCT-15 cells, human colon cancer	Ca(2+) homeostasis disruption, accumulation of H(2)O(2)	[53]
	HCT 116 cells, wild-type p53 human colorectal cancer	Upregulation of p53	[57]
	PC-3 cells, p53 mutant human prostate cancer	Negative result	[57]
	HL-60 cells, human leukemia	Increase of hydrogen peroxide	[54]
	MDA-MB-231 cells, human breast cancer	Up-regulation of Bax protein, down-regulation of Bcl-xL protein and activation of caspase-3	[58]
	H460 cells, p53-wild type human lung cancer	Up-regulation of p53 protein	[41]
	H1299 cells, p53-null human lung cancer	Mechanism was not studied	[41]
	HCT-15 cells, human colon cancer	Mechanism was not studied	[39]
DATS	MRC-5 cells, human lung fibroblast	Mechanism was not studied	[43]
	A549 cells, human lung cancer	Mechanism was not studied	[43]
	BGC823 cells, human gastric cancer	G1/S arrest	[45]
SAC	Male Syrian hamsters 7,12-DMBA-induced buccal pouch (HBP) carcinogenesis	Inhibition of Bcl-2	[36]
	SW-480 and HT-29 cells	Negative result	[40]
SMAC	SW480 cells, human colon cancer	Microtubule depolymerization and JNK1 activation	[56]
	NIH3T3 cells, mouse fibroblast	Microtubule depolymerization and JNK1 activation	[56]
	SW-480 and HT-29 cells, human colon cancer	Increase in caspase-3-like activity, induction of jun kinase activity and reduction of glutathione	[40]
	HEL and OCIM-1 cells, human erythroleukemia	Mechanism was not studied	[150]
Allicin	SGC-7901 cells, human gastric cancer	Mechanism was not studied	[151]
	L-929 cells, murine fibrosarcoma	Activation of caspases-3, -8 and -9 and cleavage of PARP	[152]
	SW480 cells, human colon cancer	Activation of caspases-3, -8, -9 and cleavage of PARP	[152]
	SiHa cells, human cervical cancer	Activation of caspases-3, -8, -9 and cleavage of PARP	[152]
	SGC-7901 cells, human gastric cancer	Inhibition of telomerase activity	[151]
	Hela cells, human cervical cancer	Activation of caspases-3, -8, -9 and cleavage of PARP	[152]
	HL-60 cells, human leukemia	Mechanism was not studied	[153]
Ajoene	B16F10 cells, murine melanoma	Activation of caspase-3	[64]
	Basal cell carcinoma tumor patients	Decrease of Bcl-2 expression	[65]
	TE354T cells, basal cell carcinoma	Mitochondria-dependent route of apoptosis	[65]
	HL-60 cells, human promyeloleukemic	Activation of MAPKs (JNK, p38 and ERK1/2) as well as the survival kinase Akt. JNK activation was not dependent on ajoene-induced mitochondria perturbation and subsequent caspase activation	[63]
	HL-60 cells, human leukemia	Activation of caspase-3 and the cleavage of the antiapoptotic protein Bcl-2. Reactive oxygen species-dependent pathway leading to caspase-dependent Bcl-2 cleavage	[62]
	HL-60 cells, human leukemia	ROS modulated apoptosis, activation of nuclear translocation of the transcription factor nuclear factor kappaB,	[44]

Table 1 (*Continued*)

GCs	Cell lines/animal model	Mechanisms/remarks[a]	Reference
	HL-60, U937, HEL and OCIM-I cells, human leukaemia	Reduction of the bcl-2-expression, increase in the activation of caspase-3 level	[154]
	HL-60 cells and blood cells from a leukemic patient, human leukaemia	Activation of caspase-3-like activity as well as to the proteolytic processing of procaspase-3 and -8; release of cytochrome *c*, which was not inhibited by zVAD-fmk, mitochondria-dependent caspase cascade which includes also the activation of the initiator caspase-8	[46]
Garlic extract	Hamsters, 7,12-dimethylbenz[*a*]anthracene-induced buccal pouch carcinogenesis	TG and inhibition of Bcl-2 expression	[36]

[a] If negative results are not specified, default results are positive.

that the biological effects of GCs are associated with their reactivity with cysteine and GSH.

Lower cytotoxicity in normal cells compared to cancer cells is a prerequisite for any chemopreventive agent. Heretofore, only a few studies compared apoptosis induction by GCs in non-neoplastic cells and neoplastic cells. Sakamoto et al. [43] showed that DATS effectively inhibited cell proliferation and induced apoptosis in human lung cancer A549 cells but not in non-neoplastic lung MRC-5 cells. Dirsch et al. [44] also reported ajoene-induced apoptosis in the human acute myeloid leukemia cell line HL-60 as well as in peripheral blood mononuclear cells (PBMCs) isolated from a chronic leukemia patient but quiescent and proliferating PBMCs isolated from healthy donors remained unaffected. However, the data are still very rudimentary and the mechanisms responsible for this differential effect remain unknown. Therefore, further investigations are required in this direction before GCs are promoted as safe chemopreventive agents.

Generally, most GCs inhibit cell cycle of cancer cells at G2/M phase and induce apoptosis via the mitochondria pathway, which constituted of modulation of bcl-2 family, change in mitochondria membrane potential and cytochrome *c* release. However, Li and Lu [45] reported that DATS induce G1/S cell arrest and apoptosis in gastric cancer cells. So far, data are not available regarding the involvement of the death receptor pathway in GCs-induced apoptosis. Dirsch et al. [46] reported ajoene-induced apoptosis in leukemic cell with functionally inactive CD95 receptor. FasL binding is supposed to induce Fas trimerization and recruits initiator caspase-8 via the adapter protein FADD [47]. The results of these authors suggested that CD95 death receptor was not involved in death signaling induced by ajoene. However, unpublished data from our lab showed upregulation by DADS of expression of CD95 receptor in human lung cancer A549 cells, which resulted in activation of caspase-8. Therefore, future studies are needed to reveal the role of cell death receptors in GCs-induced apoptosis. JNK activation and intracellular redox environment have been suggested to modulate so many physiological or pathological processes such as Parkinson disease, cancer, cell differentiation and apoptosis [48,49]. Modulation of intracellular oxidative stress has been reported during apoptosis induction by some naturally occurring chemopreventive agents such as capsaicin, green tea polyphenols, and epigallocatechin-3-gallate (EGCG) [50–52]. Likewise, reactive oxygen species (ROS) production and JNK activation seem to be initiators of GCs-induced apoptosis. Increase of ROS and activation of JNK have been found in DADS, ajoene, and SAMC-induced apoptosis in various human cancer cells [53–56]. However, the role of JNK in apoptosis signaling seems variable in different cells, which is discussed in the following sections.

Among the GCs, apoptosis induced by DADS and ajoene were extensively studied and will be reviewed separately in following sections.

2.1. Dially disulfide

Diallyl disulfide (DADS) is an oil-soluble sulfur compound from garlic that is produced as a result of decomposition of allicin. About 60% of garlic oil was reported to be DADS, indicating that it is the most prevalent oil soluble garlic constituent.

DADS induced apoptosis in human colon tumor cells (HCT-15 and HCT-116) [39,53,57], non small cell lung cancer cells (NSCLC) H1299 [41], human breast cancer cell lines (KPL-1, MCF-7, MDA-MB-231 and MKL-F) [58], human leukaemia HL-60 cells [54] and neuroblastoma cells [55]. However, in human prostate PC-3 cells, DADS failed to induce apoptotic effects [57]. Furthermore, results from the same lab showed that DADS and DATS could reduce growth of A-549 cells, a neoplastic lung cell line, and MRC-5 cells, a non-neoplastic cell line, the effect in the former being much stronger than the latter [43]. This differential effect requires further investigation as the mechanism behind it is not known and it is unclear whether non-tumor cells are more resistant than tumor cells to the apoptotic effect of the compound.

Apoptosis induction by DADS involves different apoptotic genes and enzymes depending on the cell type used in the study. Generally, up-regulation of bax, down regulation of bcl-2 and bcl-XL, cytochrome *c* release, activation of caspase-3 and caspase-9, increase of intracellular free calcium, cleavage of caspase-3 and PARP have been reported [54,55,58]. These findings suggest that DADS-induced apoptosis is dependent on the classical mitochondria pathway. DADS treatment increased intracellular H_2O_2 and other ROS within less than 30 min, indicating that ROS formation is the earliest event in the cascade of apoptosis induction [53,55]. Addition of free radical scavengers such as *N*-acetylcysteine (NAC) and catalase abrogated apoptosis induced by DADS [53], suggesting that oxidative stress is perhaps the triggering factor in DADS-induced apoptosis. Redox sensitive gene, JNK, was also activated by DADS [55]. A specific JNK inhibitor, JNK inhibitor I, significantly reduced DADS-induced apoptosis indicating that JNK activation is necessary for DADS-induced apoptosis. P53 is recognized as a key molecule in the induction of cell death. Bottone et al. [57] reported that DADS induced p53 and NAG-1 in a dose-dependent manner in wild type p53 cell line and HCT-116, but not in p53 mutant cell lines HCT-15 and PC-3. Thus, DADS-induced apoptosis and NAG-1 protein expression appear to occur via a p53-mediated pathway. In another study, DADS-induced apoptosis in p53 wild type H460 cells was accompanied by an increase in p53 level, but DADS also induced apoptosis in p53-null type H1299 cells [41]. These reports indicate that the role of p53 in DADS-induced apoptosis is cell type dependent. Additionally, increase in the intracellular calcium concentration seems to be another triggering factor for apoptosis induction by DADS [39,53]. In these studies, DNA fragmentation induced by DADS was completely blocked by intracellular Ca^{2+} chelator.

2.2. Ajoene

Ajoene is a garlic-derived compound produced from pure allicin and has the advantage of a greater chemical stability than allicin. Ajoene inhibited aflatoxin B1 [59], benzo[*a*]pyrene- and 4-nitro-1,2-phenylenediamine-induced [60] mutagenesis in vitro and blocked mouse skin tumor formation induced by 12-*O*-tetradecanoylphorbol-13-acetate in vivo [61].

Ajoene has been reported to induce apoptoisis in human leukemia cell lines HL-60 [46,62,63], murine melanoma B16F10 cells [64], basal cell carcinoma cell line TE354T and primary basal cell carcinoma cells [65]. Basically, ajoene-induced cell death is linked to mitochondrial membrane permeabilization (MMP), the release of cytochrome *c* and generation of reactive oxygen species (ROS) but seems to be independent of Fas/Caspase-8 pathway [44,62]. Additionally, unlike with other garlic constituents, induction of intracellular ROS by ajoene seems not to depend on mitochondrial perturbation and is upstream of caspase activation because of no significant difference between HL-60/neo and HL-60/bcl-xL cells regarding their ROS response after ajoene exposure [44,46]. This is unclear and may be untrue. DADS induced ROS within 15 min and reached a peak level within 30 min. Furthermore, Antlsperger et al. [63] found that ajoene induced the activation of JNK, p38 and extracellular signal-regulated kinases (ERK)1/2 as well as the survival kinase Akt. However, introduction of the corresponding inhibitors indicated that apoptosis triggered by ajoene does not require activation of JNK and p38 MAPK which are activated by cellular stress in HL-60 cells. But apoptosis could be amplified by inhibition of ERK. Stimulation of Akt, the known survival factor, by ajoene did not result in the same effect as did ajoene-activated ERK. Addition of ERK inhibitor attenuated apoptosis suggesting that cell death and survival signaling collectively participated in programmed cell death induced by ajoene. As activation of mitogen-activated protein kinases

(MAPKs) is considered to be a key step in ROS-induced signaling pathways, this study suggested that ROS influence ajoene-induced apoptosis via modulation of ERK but not JNK and p38 MAPK. Since JNK-dependent apoptosis is induced by DADS [55], JNK may play different roles in apoptosis induced by different garlic constituents.

3. Apoptosis induction by isothiocyanates (ITCs)

Isothiocyanates (ITCs) are hydrolysis products of a group of naturally occurring thioglucoside compounds, glucosinolates, found in cruciferous vegetables such as watercress, Brussels sprouts, broccoli, cabbage, kai choi, kale, horseradish, radish and turnip [66]. Therefore, ITCs are widely consumed by humans. Many ITCs are effective chemopreventive agents against carcinogen-induced cancers in experimental animals. ITCs inhibit cancer formation or reduced cancer growth in various tissues such as rat lung [67–69], esophagus [70–72], mammary gland [73,74], liver [75,76], small intestine [77], colon [78], pancreas [79], and bladder [80,81]. The chemical structures of common ITCs (allyl isothiocyanate (AITC), benzyl isothiocyanate (BITC), phenethyl isothiocyanate (PEITC) and sulforaphane (SFN)) are shown in Fig. 3.

Previous studies suggested that ITCs exert chemopreventive effects via inhibition of carcinogen activation, enhancement of the detoxification and excretion of carcinogens through induction of detoxifying phase II enzymes such as glutathione-*S*-transferase (GST), quinone reductase, epoxide hydrolase, and UDP-glucuronosyltransferase (see the review by Conaway et al. [66]).

In recent years, chemopreventive mechanisms dealing with retardation or inhibition of the develop-

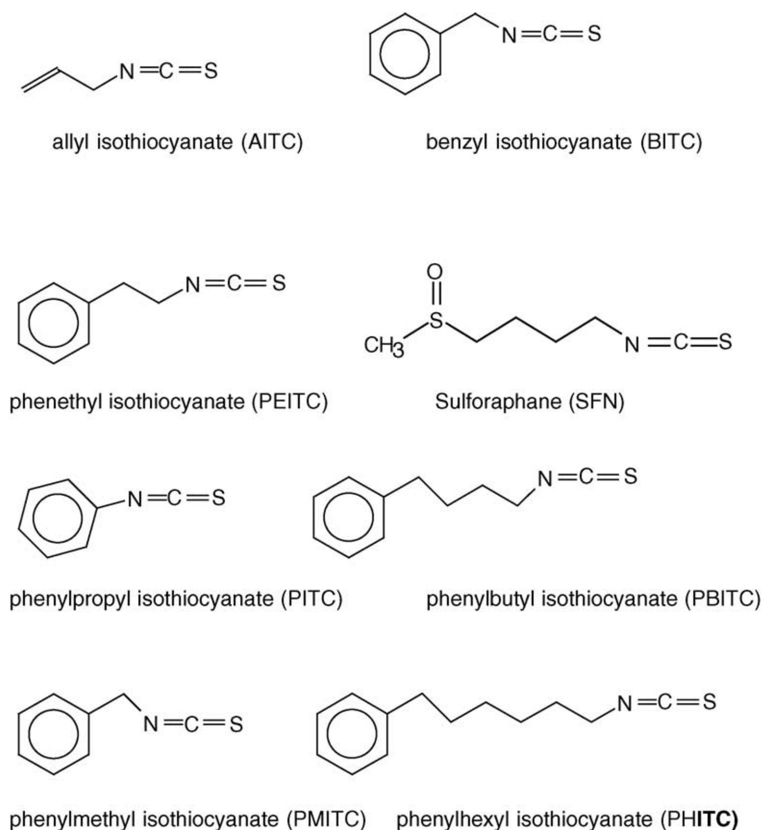

Fig. 3. Chemical structure of ITCs.

ment of neoplastic cells attracted considerable attention. In this regard, apoptosis induced by various ITCs was extensively studied in cancer cell lines originating from various tissues. Some data from animal studies are also available (Table 2). Besides the effect of individual ITC compounds, *Brassica* vegetable juices also showed apoptosis induction in rat colonic mucosal crypts in vivo [82]. In an earlier work from same group, the precursor of AITC, sinigrin, also induced apoptosis in rat colon aberrant crypt foci [83]. However, not all ITCs induce apoptosis. For instance, 6-phenylhexyl isothiocyanate (PHITC) failed to induce apoptosis in colon cancer in the rats [84].

Apoptosis-inducing potency of ITCs seems to be responsible, at least in part, for their chemopreventive efficacy in some animal models. For example, PHITC, which was the most potent inhibitor of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumors in F344 rats and A/J mice was also the most potent apoptosis inducer in HeLa cells. On the contrary, PITC, which showed minimal anticarcinogenic activity as compared to other ITCs, did not induce apoptosis [85].

The potency of apoptosis induction by ITCs is different and seems to be greatly influenced by the chemical structure of the compound as well as the cell under study. For instance, 10 μM of SFN demonstrated much stronger potential to induce caspase-3 activity compared to equal concentrations of PEITC and BITC [86]. Moreover, the kinetics of caspase-3 activation among different ITC compounds was quite divergent and peak activation was observed at different times: 12 h for PEITC and 48 h for SFN [87]. In another study, it was shown that whereas BITC, the predominant ITC compound in garden cress, induced apoptosis and MAPK activation in head and neck squamous cell carcinoma [88], its structural analog, PITC, which lacks a $-CH_2-$ spacer is devoid of these effects. So far, it is not known if such structure-related biological effect differences exist among other ITCs as well. Additionally, one study revealed that AITC, the predominant ITC compound in Brussels sprouts, significantly inhibits survival of PC-3 and LNCaP human prostate cancer cells, whereas proliferation of a normal prostate epithelial cell line is minimally affected even at concentrations that are highly cytotoxic to the prostate cancer cells [89]. Taken together, induction of apoptosis in cancer cells by ITCs seems to be a universal biological phenomenon for ITCs and the action may contribute to chemopreventive effect of these groups of compounds.

ITCs may accumulate rapidly to very high concentrations (several hundred-fold over the extracellular concentrations) in vitro, that the degree of such accumulation was closely correlated with their potencies in inducing phase II detoxification enzymes (glutathione transferases and quinone reductase) in murine hepatoma Hepa cells and that those ITCs that did not accumulate were not inducers [90]. Further study showed that intracellular conjugation of ITCs with GSH is mainly responsible for ITC accumulation [91]. This may result in depletion of GSH and an increased intracellular concentration of ROS (see Table 2). As ROS may act as signaling molecules in different biological processes including initiation and execution of apoptosis [92], modulation of the intracellular redox environment may be pivotal in ITC-induced apoptosis. Among oxidative stress-induced signaling proteins, MAP kinases are the most important ones. In particular, ITC-induced activation of MAPKs has been shown to play an important role in the regulation of transcription of downstream genes such as AP-1, NF-κB and other biological processes including apoptosis. One recent study in HT-29 human colon cancer cells demonstrated that PEITC, SFN and AITC inhibited activation of lipopolysaccharide (LPS)-induced NF-κB expression [86]. NF-κB plays an important role in signal transduction pathways of cancer and chronic inflammatory diseases [93] and it protects cancer cells from apoptotic stimuli [94]. Therefore, inhibition of NF-κB by ITCs may represent a proapoptotic signal transduction pathway. Kong et al. [95] proposed a pattern of signal transduction events that are affected by ITCs and other natural chemopreventive agents. According to these authors, at low concentrations as activation of MAPKs pathway may lead to the induction of genes such as c-Fos, c-jun, GST, and QR resulting in protection and survival mechanisms. Increasing concentrations of the agents will also activate the MAPK pathway; however, the ICE/Ced 3 protease (caspases) pathway will also be activated concomitantly, which will lead to apoptotic cell death. The aforementioned proposal was supported by studies in which 5–10 μM PEITC strongly activated JNK in a time and concentration

Table 2
Apoptosis induction by ITCs

ITCs	Cell lines/animal models	Mechanisms/remarks[a]	Reference
AITC	HL60 ($p53^-$) and human myeloblastic leukaemia-1 cells ($p53^+$), human leukaemia	Caspase-8 activation; apoptosis is not p53 status dependent	[120]
	HT-29 cells, human colorectal cancer	Mechanism was not studied	[155]
	HL-60 cells, human leukemia	Cleavage of BID protein, tyrosine phosphorylation and activation of JNK	[109]
	PC-3 cells, human prostate cancer xenografts in mice	Reduction of Bcl-2 and cleavage of Bid	[156]
	PC-3 cells, human prostate cancer	G(2)/M arrest and decrease in Bcl-2 and, Bcl-X(L)	[89]
	LNCaP cells, human prostate cancer	Decrease in the levels of proteins that regulate G(2)/M progression, decrease of Bcl-X(L) protein expression	[89]
BITC	BxPC-3 cells, human pancreatic cancer	Increase of Bax/Bcl-2 ratio, activation of procaspase-3 and PARP and inhibition of NF-κB	[157]
	HT29 cells, human colon cancer	Activation of caspases-3	[149]
	Mouse, lung cancer induced by benzo(A)pyrene	MAPKs activation, activator protein-1 (AP-1) activity increased and p53 phosphorylation	[69]
	Jurkat cells, human T-cell leukemia	Activation of JNK and p38 MAPK but not ERK; Bcl-2 protein was phosphorylated without alteration of the Bcl-2 total protein amount	[158]
	HNSCC cells, head and neck squamous cell carcinoma	Activation of caspase-3 and PARP cleavage; activation of p38 MAPK and p44/42 MAPK, corresponding inhibitors partly abrogated apoptosis	[88]
	RL34 cells, rat liver epithelial	Caspase-3 activation, release of cytochrome *c*, glutathione depletion by diethyl maleate significantly accelerated BITC-triggered apoptosis	[159]
	LS-174 and Caco-2 cells, human colon adenocarcinoma	Mechanism was not studied	[127]
PEITC and its metabolites	DU-145 cells, human prostate cancer	G0/G1 arrest and enhancement of p21 protein	[160]
	LNCaP cells, human prostate cancer	G0/G1 arrest and enhancement of p21 protein	[160]
	HT-29 cells, human colon cancer	Inhibition of NF-κB activity and activation of caspase-3	[86]
	HL-60 cells, human leukaemia	Inhibition of protein kinase C (PKC)	[161]
	HL60 ($p53^-$) and human myeloblastic leukaemia-1 cells ($p53^+$), human leukaemia	Caspase-8 activation and); apoptosis is not p53 status dependent	[120]
	HL60 cells, human leukaemia	Cleavage of Bid and JNK activation	[109]
	A/J Mouse, lung cancer induced by benzo(A)pyrene	MAPKs activation, activator protein-1 (AP-1) activity increased and p53 phosphorylation	[69]
	HepG2 cells, human liver cancer cells	Increase of superoxide, but radical scavengers failed to block apoptosis	[162]
	HCT-116 cells and HCT15 cells, human colon adenocarcinoma	Induction of GADD153 gene independence of p53	[122]

Table 2 (*Continued*)

ITCs	Cell lines/animal models	Mechanisms/remarks[a]	Reference
	T24 and Jurkat cells, human bladder carcinoma and human leukemia	JNK, ERK and p38 are activated but inhibitors of them did not block apoptosis; depletion of cellular glutathione	[110]
	Mouse, immunodeficient mice with xenografted tumors	Mechanism was not studied	[108]
	HT-29 cells, human colon adenocarcinoma	Cytochrome *c* release, JNK, ERK and p38 activation. JNK inhibitor abrogated apoptosis	[114]
	HL-60 cells, human leukaemia	Mechanisms was not studied	[163]
	HL60 and ML-1 cells, human leukaemia	GSH depletion and thiocarbamoylation	[109]
	HeLa cells, human cervix adenocarcinoma	Caspase-3-like protease activity	[85]
	JB6 cells, mouse epiderm	p53-dependent pathway	[115]
	LNCaP cells, human prostate cancer	JNK activation and apoptosis in prostate cancer cell lines with distinct p53 status. PEITC induced JNK-mediated apoptotic signaling via a different pathway than that used by DNA-damaging agents	[121]
	PC-3 cells, human prostate cancer	p53 is not required for PEITC-induced apoptosis in the PC-3 human prostate cancer cell line and that the PEITC-induced apoptosis is mediated by extracellular signal-regulated kinases (ERK1/2)	[112]
	Various cell types	JNK mediated apoptosis; oxidative stress triggered apoptosis; bcl-2 and bcl-xL were involved but are upstream of JNK	[100]
PMITC	Various cell types	JNK mediated apoptosis; oxidative stress triggered apoptosis; bcl-2 and bcl-xL were involved but are upstream of JNK	[100]
	HeLa cells, human cervix adenocarcinoma	Induction of caspase-3-like protease activity	[85]
PBITC	HeLa cells, human cervix adenocarcinoma	Induction of caspase-3-like protease activity	[85]
PHITC	HeLa cells, human cervix adenocarcinoma	Induction of caspase-3-like protease activity	[85]
	Male F344 rats	Negative result	[84]
PITC	HeLa cells, human cervix adenocarcinoma	Negative result	[85]
	HNSCC) cells, head and neck squamous carcinoma	Negative result	[88]
2-Oxohexyl isothiocyanate	L-1210 cells, mouse leukemia	Mechanism was not studied	[125]
SFN and its metabolite	Caco-2 cells, human colon cancer	Activation of ERK1/2	[129]
	HT-29 cells, human colon cancer	Inhibition of NF-κB activity and activation of caspase-3	[86]
	HT29 cells, human colon cancer	Increased expression of bax and release of cytochrome *c*	[128]
	LS-174 and Caco-2 cells, human colon cancer	Mechanism was not studied	[127]
	LNCaP cells, human prostate cancer	Expression of cyclin D1 and DNA synthesis were inhibited	[131]
	Human T lymphocytes	Increased expression of p53 and decrease of cyclin D3	[97]
	L-1210 cells, mouse leukemia	Mechanism was not studied	[125]
	F3II cells, BALB/c mouse mammary cancer	Bcl-2 down regulation	[133]

Table 2 (*Continued*)

ITCs	Cell lines/animal models	Mechanisms/remarks[a]	Reference
	HepG2 cells, human liver cancer	GSH depletion and the caspase-3 activity blocked by exogenous GSH; JNK1/2 activated and blocked by exogenous GSH	[132]
	DU-145 cells, human prostate cancer	Down-regulated bcl-2 and Rb	[96]
	DAOY cells, human metastatic medulloblastoma	Activation of caspases-3 and -9	[87]
	Transformed and non-transformed human T lymphoctyes	Increased expression of p53 and bax protein, slight change of bcl-2 in transformed cells; increase of p53 but a little effect on bcl-2 and bax in non-transformed cells	[98]
	PC-3 cells, human prostate cancer	Caspase-8 and -9 activation	[130]
	Jurkat cells, human leukemia	G(2)/M-phase arrest, increased p53 and bax protein expression, slight change ofbcl-2 expression	[97]
	LS-174 and Caco-2 cells, human colon cancer	Mechanism was not studied	[127]
Glucosinolate sinigrin	Rat	Mechanism was not studied	[83]
Freeze-dried raw and microwave-cooked Brussels sprouts	Rat	Mechanism was not studied	[82]

[a] If negative results are not specified, default results are positive.

dependent manner whereas increasing the concentrations of PEITC from 10 to 20 μM strongly activated caspase-3-like protease activity in HeLa cells [85]. The above pattern is based on the evidence from limited cell models and certain agents and it is not known if such dose-dependent differential transcription of MAPK-regulated genes is a common feature for other ITCs. However, it seems logical to say that lower ITC concentrations cause only a mild oxidative stress, which may stimulate the cell to build up its antioxidant defence, whereas higher doses result in irreversible changes, which result in apoptosis. In line with this, Chen et al. reported that treatment of cells for a short period with ultraviolet C and gamma radiation led to transient activation of JNK and cell proliferation, whereas extended treatment with the same agents lead to sustained JNK activation and apoptosis. Considering the existences of multiple signal transduction pathways involved in the apoptotic effect of ITCs and the complicated cross-talk between different pathways, further studies are needed to elucidate the influence of ITC structure and concentration or tissue origin of the cell studied on apoptosis induction by these compounds.

Accumulating evidence indicates that the mitochondrial pathway plays a pivotal role in the apoptotic effect of ITCs. Modulation of the bcl-2 family of genes consisting of proapoptotic and antiapoptotic proteins is an important part of the mitochondria-mediated apoptosis and has been extensively observed in ITC-induced apoptosis as summarized in Table 2. ITCs activate bax but suppress bcl-2 and bcl-xl. However, modulation of bcl-2 in ITCs-induced apoptosis is cell model-dependent. For example, SFN resulted in the down-regulation of bcl-2 in human prostate cancer cells [96]. On the contrary, Bcl-2 was slightly affected or unaffected during apoptosis induction by SFN in human T lymphocytes and leukemia cells [97,98]. Additionally, inhibition of apoptosis by anti-apoptotic Bcl-2 and Bcl-x(L) is associated with protection against ROS and/or a shift of the cellular redox potential to a more reduced state [99,100], implying a cross-talk between bcl-2 family and other signaling molecules. Not only antioxidants, but bcl-2 also may suppress PEITC-induced JNK activation and apoptosis [100], indicating that bcl-2 is an upstream suppressor for JNK activation by apoptotic stimuli. Overall, mitochondria are involved in the apoptotic

effect of most ITCs, but the exact mechanisms and possible cross-talk with other signal transduction pathways are far from being elucidated.

Among ITCs, PEITC and SFN received the most intensive attentions and will be discussed in following separated sections.

3.1. Phenethyl isothiocyanate

PEITC exists in nature as gluconasturtiin; In particular high levels are found in watercress [101]. Among ITCs, PEITC has been extensively investigated for its chemopreventive action. PEITC is able to inhibit chemically induced lung, forestomach and esophageal tumorigenesis effectively [102–106]. Chemopreventive action of PEITC was assumed to be associated with inhibition of metabolic activation of carcinogens by phase I enzymes and increased excretion of carcinogen through induction of activities of quinone reductase and GST [107].

PEITC-induced apoptosis has been reported in cancer cells of the blood, lung, liver, colon, bladder, prostate and skin (as summarized in Table 2). Two in vivo studies also showed that NAC conjugate of PEITC induced apoptosis in mice with xenografted tumors of human prostate cancer PC-3 cells and benzo(*a*)pyrene-treated mice that subsequently developed lung cancer [69,108].

The mechanism of PEITC-induced apoptosis is not clear, but available studies suggest that it is closely associated with induction of oxidative stress, activation of MAPKs, and dysfunction of mitochondria. It was reported that PEITC may deplete cells of GSH by formation of their GSH conjugates in human leukemia cells [109,110]. GSH efflux is regarded as a critical early event in the execution of apoptosis [111]. Several studies showed the involvement of MAPKs in PEITC-induced apoptosis. Although three members of MAPKs, JNK, ERK1/2 and p38, are activated by PEITC [100,110,112,113], the role of the individual MAPK in PEITC-induced apoptosis varies depending on the cell line used. For example, abrogation of PEITC-induced apoptosis in HT-29 cells by a specific JNK inhibitor suggests that JNK is indispensable for the apoptotic effect of PEITC [114]. PEITC but not PITC or PPITC induced sustained JNK activation in human leukemia Jurkat cells. Also, not PITC and PPITC but PEITC caused DNA laddering with the same concentrations in the same cell line [100]. These results indicate the correlation between persistent activation of JNK and apoptosis induction by ITCs. Furthermore, caspase inhibitors failed to inhibit JNK activation suggesting that JNK activation initiates apoptotic signaling [100]. On the contrary, not JNK but ERK1/2 and p38 were activated by PEITC-induced apoptosis in PC-3 human prostate cells. Furthermore, ERK's inhibitor but not p38's inhibitor abolished PEITC induced apoptosis [112]. In a recent study by Pullar et al. [110], JNK, ERK and p38 were activated by PEITC in human leukemia cells but their inhibitors did not block apoptosis. The above studies suggest that activation of MAPKs is a common event in ITCs-induced apoptosis, but the role of individual member of MAPKs is cell type dependent.

As with MAPKs, the role of p53 in the apoptotic effect of PEITC is cell line dependent. Huang et al. reported that apoptosis induction by PEITC is mediated through a p53-dependent pathway, which was corroborated by PEITC-induced apoptosis in $p53^+$ cells but not in $p53^-$ mouse embryo fibroblasts cells [115]. It is well known that p53 not only a key player in apoptosis cascade but also play a important role in DNA damage and DNA-repair. Namely, p53 will induce cell cycle arrest and even apoptosis when the DNA damage is heavily and not repairable [116]. As our previous studies suggested certain genotoxicity caused by PEITC and other ITCs in vitro and in vivo [117–119], it is reasonable to speculate that p53-dependnet apoptosis may be triggered by irreparable DNA damage induced by ITCs. However, Xu and Thornalley [120] reported inhibition of growth and induction of apoptosis by PEITC in both $p53^-$ and $p53^+$ leukaemia cells. In addition, investigation of PEITC-induced apoptosis using $p53^-$ wild type, p53 deficient and mutant human prostate cancer cell lines also suggested that PEITC exerts apoptosis on human cancer cell lines with distinct p53 status [112,121]. If DNA damage is involved in p53-indepednent apoptosis induced by ITCs is unknown, one study suggested PEITC creates an oxidative cellular environment that induces DNA damage and GADD153 gene activation independence of p53, which in turn helps trigger apoptosis [122]. Overall, implications of DNA damage and p53 in ITCs mediated-apoptosis is intricated and further studies are needed to address these important issues.

3.2. Sulforaphane

The glucosinolate of SFN is contained in several cruciferous vegetables but predominantly in broccoli [66]. Previous studies demonstrated that SFN induces high levels of mammalian phase II enzymes via an antioxidant response element (ARE)-mediated transcriptional activation [123,124]. In addition, SFN reduced breast cancer incidence and minimized the size of the tumor in a rat model [124].

SFN has showed apoptosis inducing potential in human leukaemia cells [125,126], human cancer cells of colon [127–129], prostate [96,130,131], liver [132], brain [87] and skin [125] and mouse mammary cancer cells [133]. However, it did not induce caspase-3 activity in human renal Caki-1 cells, human glioblastoma U87 cells and human metastatic medulloblastoma BAEC cells suggesting that SFN-induced apoptosis is cell type dependent [87]. An in vivo study demonstrated that SFN induced apoptosis in PC-3 prostate cancer cell xenografts in mice [130].

Kim et al. [132] observed that SFN induced antioxidant response element (ARE)-related gene expression at lower concentrations but induced apoptosis and activation of JNK at higher concentration. Moreover, expression of ARE-related genes, JNK and apoptosis were blocked by addition of extracellular GSH. The study implicated the overall important roles of GSH in SFN-mediated signaling, gene expression, and apoptosis. Most studies indicated that SFN-induced apoptosis is caspase-dependent and involves caspase-3, caspase-9 and caspase-8 [87,130,132]. Although specific caspase-8 inhibitor partly abolished cleavage of procaspase-3 suggesting a role of caspase-8 in apoptosis induction by SFN [130], so far, no reports are available regarding the involvement of cell death receptors. Several studies showed that up-regulation of pro-apoptotic protein bax and down-regulation of anti-apoptotic protein bcl-2 resulted in an increase in mitochondrial permeability and subsequent cytochrome *c* release [96–98,128,133]. However, bax and Bcl-2 expression level was only slightly affected by SFN in human medulloblastoma cells, human Jurkat T-leukemia cells and human T lymphocytes [87,97,98].

The role of p53 in SFN-induced apoptosis is controversial. One study indicated that p53 expression level was not affected by SFN [128]. However, SFN markedly increased p53 expression in both human T-cell leukemia and non-transformed phytohemagglutinin-stimulated human lymphocytes [98]. SFN induced the activation of both ERK2 and JNK in HepG2 cells and increased the expression of ARE reporter gene [132,134]. However, the implication of modulation of ERK and JNK in apoptosis cascade induced by SFN is still unknown.

4. Conclusions and perspectives

In summary, the available literature indicates that GCs and ITCs target various apoptosis signaling molecules from initiation to execution as shown in a scheme of Fig. 4. These molecules include MAPKs (JNK, ERK1/2 and p38), P53, NF-κB, bcl-2 family, and caspases. But for certain ITCs or GCs, apoptosis did not involve all of the above molecules. With few exceptions, apoptotic effects of GCs and ITCs were triggered by increased intracellular production of ROS, suggesting the importance of the intracellular redox environment for apoptosis induction. Although several apoptosis signaling pathways were established, the roles of these pathways in apoptotic induction by GCs and ITCs are not known and remain a challenge for future research. In addition, GCs and ITCs may affect the expression of genes that are not yet identified but play an important role in the apoptotic effect of these compounds. Whether OSCs induce apoptosis in non-tumor cells is also a critical issue that must be addressed. In general the outstanding problems regarding the apoptotic effect of GCs and ITCs and future research directions are summarized as follows.

4.1. Apoptosis induction by OSC in non-tumor cells

One of the areas where a major gap exists regarding apoptosis induction by ITCs and GCs is the profile on the effect of the compounds in tumor cells versus non-tumor cells. Only two reports compared ITCs and GCs-induced apoptosis in normal and transformed cells (as shown in Tables 1 and 2). To evaluate the safety of GCs and ITCs in chemoprevention, further efforts are needed to investigate apoptosis induction in normal cells and the mechanisms involved.

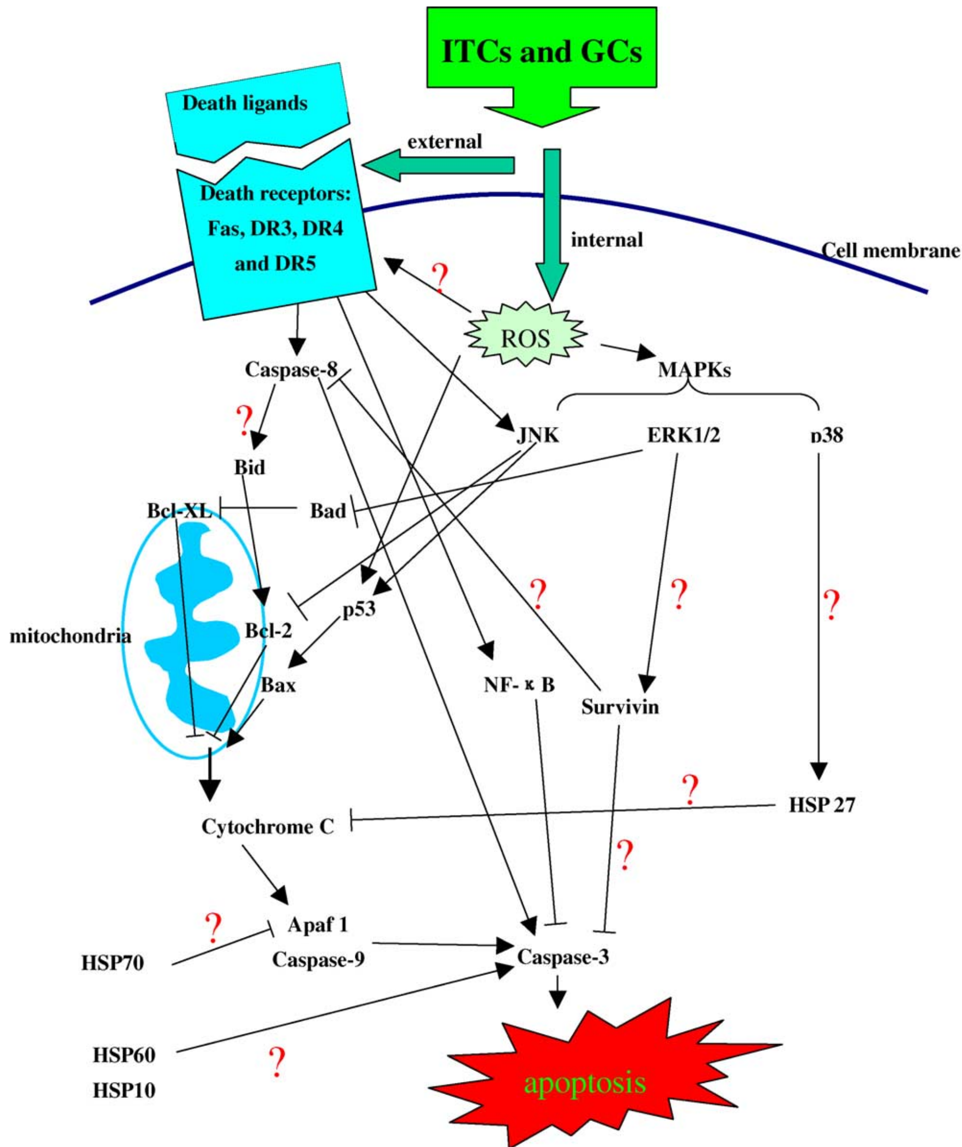

Fig. 4. A model of cell signaling pathways in GCs and ITCs modulated apoptosis. DR, death receptor; ERK1/2, extracellular signal-regulated kinase; HSP, heat shock protein; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor κ B; MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species. Question marks represent unconfirmed pathways.

4.2. The role of death receptors pathway in OSC mediated apoptosis

In addition to the intrinsic pathways of apoptosis, the extrinsic pathway involving cell death receptors and their ligands play a very important role in regulating apoptosis. Apoptosis induction by other chemopreventive agents such as all-trans-retinoic acid, sulindac and epigallocatechin gallate was reported to be mediated via the death-receptor pathway [135–137]. However, to date, there are few reports on the involvement of the extrinsic pathway in the apoptotic effects of ITCs and GCs.

4.3. Role of heat shock proteins in OSC mediated apoptosis

Expression of inducible heat shock proteins (HSP) is known to correlate with increased resistance to apoptosis induced by a range of diverse cytotoxic agents and has been implicated in chemotherapeutic resistance [138]. The modulation of apoptosis by HSPs is via multiple mechanisms. HSP27 could interfere with apoptosis by binding directly to cytochrome *c* and sequestering it from Apaf-1 [139,140]. Another study revealed HSP27 controls apoptosis by regulating Akt activation with involvement of p38 [141]. HSP70 may exert its anti-apoptotic function by negative regulation of Apaf-1 apoptosome [142]. Other heat-shock proteins, HSP60 and HSP10, can be released from mitochondria and accelerate activation of caspase-3 [143]. An early study has reported that heat shock protected the human colon cancer cells from curcumin-induced apoptosis by blocking the release of apoptosis-inducing factor and activation of caspase-3 and caspase-9 [144]. Considering the facts that cells or tissues from a wide range of tumors have been shown the ability to express high levels of one or more HSPs, it is reasonable to investigate the possible of involvement of HSPs in OSC-mediated apoptosis.

4.4. The role of survivin in OSC-mediated apoptosis

Survivin is a novel member of inhibitor of apoptosis (IAP) protein family, which is aberrantly expressed in cancer but undetectable in normal, differentiated adult tissues. Current studies suggest that survivin is implicated in both control of apoptosis and regulation of cell division [145]. Previous studies revealed down regulation of expression of survivin by chemopreventive agents such as resveratrol, sulindac and celecoxib [146–148]. However, no report is available on the role of survivin in apoptosis induced by OSC.

The main problem in the elucidation of all signaling pathways that are involved in apoptosis induction by ITCs and GCs is the existence of cross-talks between different molecules, which makes the apoptosis cascade intricate. Further studies are needed to clarify these issues.

Overall, it is well established that ITCs and GCs affect xenobiotic metabolizing enzymes in such a way that carcinogens are less activated or detoxified and excreted rapidly or that DNA damage is circumvented. Together with recent findings showing that GCs and ITCs exert strong apoptosis induction potential on initiated cells, it is indicated that GCs and ITCs affect the process of carcinogenesis at multiple stages. Mechanism knowledge of the cancer chemopreventive effect of ITCs and GCs sheds more light not only on the beneficial effects to humans of garlic and cruciferous vegetables, but may also pave the way for the development of GCs and ITCs for dietary supplementation or even cancer therapeutic drugs.

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