Arch Pharm Res Vol 23, No 1, 1-16, 2000

Signal Transduction Events Elicited by Natural Products: Role of MAPK and Caspase Pathways in Homeostatic Response and Induction of Apoptosis

Ah-Ng Tony Kong[1], Rong Yu[1], Chi Chen[1], Sandhya Mandlekar[1], and Thomas Primiano[2]

[1]Center for Pharmaceutical Biotechnology, Department of Pharmaceutics and Pharmacodynamics, College of Pharmacy and [2]Department of Molecular Genetics, College of Medicine, University of Illinois at Chicago, Chicago, IL 60607, USA

(Received December 21, 1999)

Many natural products elicit diverse pharmacological effects. Using two classes of potential chemopreventive compounds, the phenolic compounds and the isothiocyanates, we review the potential utility of two signaling events, the mitogen-activated protein kinases (MAPKs) and the ICE/Ced-3 proteases (caspases) stimulated by these agents in mammalian cell lines. Studies with phenolic antioxidants (BHA, tBHQ), and natural products (flavonoids; EGCG, ECG, and isothiocyanates; PEITC, sulforaphane), provided important insights into the signaling pathways induced by these compounds. At low concentrations, these chemicals may activate the MAPK (ERK2, JNK1, p38) leading to gene expression of survival genes (c-Fos, c-Jun) and defensive genes (Phase II detoxifying enzymes; GST, QR) resulting in survival and protective mechanisms (homeostasis response). Increasing the concentrations of these compounds will additionally activate the caspase pathway, leading to apoptosis (potential cytotoxicity). Further increment to suprapharmacological concentrations will lead to nonspecific necrotic cell death. The wider and narrow concentration ranges between the activation of MAPK/gene induction and caspases/cell death exhibited by phenolic compounds and isothiocyanates, respectively, in mammalian cells, may reflect their respective therapeutic windows *in vivo*. Consequently, the studies of signaling pathways elicited by natural products will advance our understanding of their efficacy and safety, of which many may become important therapeutic drugs of the future.

Key words: MAPK, Caspases, Chemopreventive agents, Phase II drug metabolizing enzymes, Apoptosis

INTRODUCTION

Many natural products elicit diverse pharmacological / biological responses such as anti-inflammatory, anti-platelet, cardiovascular protection, chemotherapeutic and chemopreventive effects. However, the cellular signal transduction events produced by many of these agents are not well characterized and poorly understood. Using two classes of potential chemopreventive compounds the phenolic compounds / antioxidants and the isothiocyanates, we would like to review the potential utility of two signaling events, the mitogen-activated protein kinases (MAPKs) and the ICE/Ced-3 family proteases (caspases) stimulated by these agents in many human cell lines. The biological consequences of the activation of the MAPK signaling pathway / gene expression versus the caspase pathway / cell death, could be potentially important to predict the pharmacological responses in the enhancement of cell survival or apoptotic cell death and potential cytotoxicity effect.

Correspondence to: Ah-Ng Tong Kong, Center for Pharmaceutical Biotechnology MC 870 Department of Pharmaceutics and Pharmacodynamics, College of Pharmacy, University of Illinois at Chicago, 900 S. Ashland Ave MC870, Chicago, IL 60607, USA
E-mail: KongT@uic.edu

Many natural products are potent cancer protective agents

The potent anticarcinogenic effects of phenolic antioxidants that are widely used as preservatives in processed foods were first reported by Wattenberg and coworkers (Wattenberg, 1973; Wattenberg, 1977; Wattenberg *et al.*, 1977; Wattenberg *et al.*, 1980; Watt-

enberg, 1983; Wattenberg, 1985; Wattenberg, 1986; Wattenberg and Bueding, 1986). 2(3)-*tert*-butyl-4-hydroxyanisole (BHA) and 3,5-Di-*tert*-butyl-4-hydroxytoluene (BHT) were found to protect rodents against tumors formation of a variety of carcinogens. These studies were critical because they showed chemo-protection or chemoprevention by relatively benign chemicals already present in the food chain, and because of the possibility that these food additives were already providing some degree of protection in man. The list of chemicals, both synthetic and natural, were found to protect against various carcinogens began to grow. Ethoxyquin (a dihydroquinoline antioxidant used to preserve animal food), naturally occurring lactones such as coumarin, synthetic and naturally flavonoids, organic isothiocyanates, thiocarbamates (disulfiram, diethyldithiocarbamate, bisethylxanthogen), 1,2-dithiol-3-thiones, indoles and cinnamates, were all found to have chemoprevention effects in rodents and some are already minor components of human diets (Wattenberg, 1973; Wattenberg, 1977; Wattenberg· *et al.*, 1977; Wattenberg *et al.*, 1980; Wattenberg, 1983; Wattenberg, 1985; Wattenberg, 1986; Wattenberg and Bueding, 1986; Talalay, 1987; Yang *et al.*, 1992; Pezzuto, 1993; Yang and Wang, 1993; Yang *et al.*, 1994; Pezzuto, 1995; Gerha-user *et al.*, 1997; Jang *et al.*, 1997; Pezzuto, 1997; Yang *et al.*, 1998; 1999; Kim and Kim, 1999).

A large number of studies have established that isothiocyanates and the phenolic compounds / antioxidants are effective chemopreventive agents for carcinogenesis / tumorigenesis induced by a variety of carcinogens at numerous organ sites (Wattenberg, 1977; Wattenberg, 1983; Stoner *et al.*, 1991; Xu *et al.*, 1992; Katiyar *et al.*, 1993; Yang and Wang, 1993; Hecht, 1995). The isothiocyanates are potent inhibitors of eso-phageal, lung, and pancreatic tumor in rodents treated with nitrosamines and block forestomach, lung, liver and mammary tumorigenesis by polycyclic aromatic hydrocarbons (PAHs), aflatoxin, and herterocyclic amines (Hecht, 1995). Both *in vitro* and *in vivo* studies have shown that the tumor inhibitory activity of isothio-cyanates can be attributed to their ability to inhibit cytochrome P450s and/or to induce Phase II drug metabolizing enzymes involved in activation and deto-xification of carcinogens (Sparnins *et al.*, 1982a; Benson *et al.*, 1986; Bogaards *et al.*, 1990; Guo *et al.*, 1993; Zhang and Talalay, 1994; Hecht, 1995). Phenolic compounds, including green tea polyphenols (GTPs) and its major constituents, such as (-)-epigallocatechin gallate (EGCG), have been shown to effectively inhibit the tumorigenesis induced by various carcinogens in the skin, lung, forestomach, duodenum/small intestine, colon, liver, pancreas, mammary gland, and esophagus in experimental animals (Benson *et al.*, 1978; Fujita *et al.*, 1989; Wang *et al.*, 1989; Xu *et al.*, 1992; Yang and Wang, 1993; Wang *et al.*, 1994).

Mechanisms of chemoprevention

Todate, the mechanisms of action of most chemopreventive agents of carcinogenesis, both synthetic and naturally occurring, remain unclear (1999). Wattenberg has put forward three pathways by which chemopreventive agents potentially act (Wattenberg, 1983; Wattenberg, 1993): (1) Compounds that prevent the formation of carcinogens from precursor substances, examples of these include ascorbic acid (Vitamin C) and a-tocopherol (Vitamin E); (2) Compounds that act subsequent to exposures to carcinogenic agents and these have been called "suppressive agents" since they act by suppressing the expression of neoplasia in cells previously exposed to doses of a carcinogenic agent that will cause cancer; and examples of these include the retinoids, protease inhibitors, and non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin; (3) The third group of compounds that inhibit carcinogenesis by preventing carcinogenic agents from reaching or reacting with critical target sites i.e., DNA in the tissues, and these have been termed "blocking agents". These third group of agents "block" the action of carcinogens either via the induction of phase II carcinogen detoxifying enzymes in tissues such as the liver, render the carcinogens to be detoxified and subsequently excreted in the urine or bile, before they have a chance to react with DNA molecules (Wattenberg, 1983), or via blocking the phase I cytochrome P450 metabolic activation pro-cess (Yang *et al.*, 1994). As it is well established that most chemical carcinogens require metabolic activation before exerting their carcinogenic effects. The activated carcinogens are usually electrophilic agents and are highly reactive toward DNA molecules. DNA modification, especially those on oncogenes and tumor suppressor genes, are generally the driving forces for cancer development (Yang *et al.*, 1994). Hence, in theory, chemical carcinogenesis can be prevented, in most cases, either by blocking the metabolic activation process, or by induction of some detoxifying enzymes that can get rid of the carcinogens faster. Indeed, there is ample evidence to support both scenarios. Since cytochrome P450 enzymes play key roles in the activation of most carcinogens (Conney, 1982; Yang *et al.*, 1994), the inhibition of P450-dependent carcinogen activation, especially by dietary chemicals, such as diallyl sulfide (contained in garlic) and benzyl isothiocyanate (contained in many cruciferous vegetables) have been documented (reviewed in (Yang *et al.*, 1994)).

Chemopreventive agents induce gene expression of phase II detoxifying enzymes

Chemopreventive agents that are effective by virtue of inducing increases in activity of carcinogen-detoxify-

ing systems are of particular interest because they have the capacity to inhibit a wide range of carcinogens. There are two general classes of these group of chemopreventive agents (Wattenberg, 1983). The first group of compounds induce an increase in Phase II enzymes, i.e., conjugating enzymes such as glutathione S-transferases (GST), NAD(P)H: quinone oxidoreductase (NQO)/quinone reductase (QR), UDP-glucuronosyltrans-ferases (UGT), epoxide hydrolases (EH), and γ-glutamyl-cysteine synthetase (GCS) [e.g, the most extensively studied phenolic antioxidant, BHA] (Lam *et al.*, 1980). The second group of compounds induce both Phase II enzymes as well as certain Phase I enzymes such as cytochrome P450 1A1, [e.g., β-naphthoflavone] (Prochaska and Talalay, 1988). These latter group of chemicals may be complicated in that the microsomal P450 enzyme system can both activated and detoxified chemical carcinogens, as mentioned above. However, it is the first group of inducers with seemingly unrelated diverse chemical structures, both naturally occurring and synthetic, include flavonoids, phenolic antioxidants, isothiocyanates, diterpenes, indoles, unsaturated lactones, and thiocarbamates are of great interest in chemoprevention (Wattenberg, 1973; Wattenberg, 1977; Wattenberg *et al.*, 1977; Wattenberg *et al.*, 1980; Wattenberg, 1983; Wattenberg, 1985; Wattenberg, 1986; Wattenberg and Bueding, 1986; Talalay, 1987). Coincidentally, they have been shown to be preferential inducers of several hepatic Phase 2 drug metabolizing enzyme including GST, QR, EPH and UGT (Wattenberg, 1973; Cha and Bueding, 1979; Cha and Heine, 1982; De Long *et al.*, 1985; Wattenberg, 1985; Talalay, 1987; Rushmore and Pickett, 1990; Favreau and Pickett, 1991; Li and Jaiswal, 1992; Kashfi *et al.*, 1994). Treatment of animals with BHA, and its active demethylated metabolite tert-butyl-hydroquinone (tBHQ), and other chemicals that preferentially induce Phase II xenobiotic detoxifying enzymes reduces the conversion of chemical carcinogens (e.g., aflatoxin B1 and benzo[a]pyrene) to mutagenic metabolites and enhances their detoxification and excretion with the formation of conjugated metabolites. Consequently, this treatment reduces toxicity, mutagenicity, and cacrinogenicity of various chemical carcinogens (Ulland *et al.*, 1973; Weisburger and Evarts, 1977; Batzinger *et al.*, 1978; Wattenberg, 1985; Kensler *et al.*, 1987; Talalay, 1987; Kensler *et al.*, 1992).

Induction of phase 2 detoxifying genes

Phase II drug metabolizing enzymes are superfamilies of genes which encode conjugating enzymes that conjugate xenobiotics either directly or after the Phase I metabolisms such as those mediated by cytochrome P450s (Nelson *et al.*, 1993). These include GST (Rushmore, 1994), QR (Jaiswal, 1994), UGT (Mackenzie *et al.*, 1997), SULT (Weinshilboum *et al.*, 1997), and EPH (Guenthner, 1990). Conjugation with Phase II drug metabolizing enzymes usually increases hydrophilicity and thereby enhance excretion in the bile and/or urine and consequently a detoxification effect. Although under certain conditions, conjugation with Phase II enzymes can result in activated metabolites and increase toxicity (Mulder, 1990; Glatt, 1997). Each superfamily of Phase II enzymes consists of families and subfamilies of genes encoding the various isoforms with different substrate specificity, tissue and developmental expression, as well as induction and/or inhibition of the enzymes. Furthermore, analogous to the Phase I cytochrome P450 system, interspecies as well as interindividual variations in the expression of various Phase II drug metabolizing enzymes have been reported (Williams, 1978; Kitchen *et al.*, 1979; Hengstler *et al.*, 1998; Wormhoudt *et al.*, 1999).

Although the role of Phase II conjugation in the metabolism of carcinogenic and xenobiotic compounds in humans as well as in rodents, has been appreciated for a long time; the mechanism by which some of the Phase II genes are regulated remain unclear. As described above, this situation has resulted, in part, from the fact that diverse chemicals including barbiturates, planar aromatic hydrocarbons (PAHs), phorbol esters (PMA), and many chemopreventive agents including flavonoids, phenolic antioxidants, organic isothiocyanates, diterpenes, indoles, unsaturated lactones, and thiocarbamates were all found to induce Phase II genes (Wattenberg, 1977; Prochaska and Talalay, 1988; Friling *et al.*, 1990; Rushmore, 1994). Secondly, further studies of these Phase II genes revealed the existence of several *cis*-acting regulatory elements, such as the antioxidant response element (ARE)/electrophile response element (EpRE), xenobiotic-responsive element (XRE)/aromatic hydrocarbon responsive element (AhRE), activator protein-1 (AP-1), and nuclear factor-kappa B (NF-κB) binding sites in their 5-flanking regulatory region (Prochaska and Talalay, 1988; Friling *et al.*, 1990; Jaiswal, 1994; Rushmore, 1994; Pinkus *et al.*, 1996). However, recent findings from several laboratories suggest the increasingly important role of the ARE/EpRE in the regulation of expression of some Phase II genes such as NQO1, GST, and UGT by phenolic antioxidants and other chemopreventive agents (Prochaska and Talalay, 1988; Friling *et al.*, 1990; Jaiswal, 1994; Rushmore, 1994; Buetler *et al.*, 1995; Fei *et al.*, 1996; Pinkus *et al.*, 1996; Munzel *et al.*, 1999).

Transcription activation of the electrophile or antioxidant response element (EpRE/ARE)

The identification of an enhancer element in the rat GST-P gene having a phorbol 12-*O*-tetradecanoate 13-acetate responsive element-like (TRE- or AP-1-like) sequence (Okuda *et al.*, 1989), followed by the characteri-

zation of a similar xenobiotic-responsive element controlling inducible expression by phenolic antioxidants (ARE) in the rat GSTYa (Rushmore and Pickett, 1990), an electrophilic inducers responsive element (EpRE) in the mouse GSTYa (Friling *et al.*, 1990), and the hARE in the human NQOR1 gene (Li and Jaiswal, 1992), provided a common mechanism for Phase 2 gene induction. The consensus ARE/EpRE DNA sequence GTGACNNNGC resembles that of the AP-1 DNA sequence (TGAC/GTCA) (Okuda *et al.*, 1989; Friling *et al.*, 1990; Rushmore and Pickett, 1990; Li and Jaiswal, 1992), and the MARE (Maf recognition element; TGCTAC/GTCACT/C) (Igarashi *et al.*, 1994; Kataoka *et al.*, 1994). Furthermore, the discovery of the ARE/EpRE supported previous observations that monofunctional inducers such as tert-butyl-hydroquinone (tBHQ) induced Phase II genes expression by means of an electrophilic signal which operates independently of aromatic hydrocarbon (Ah) receptors or induction/expression of CYP1A1 enzyme, as this element responds to tBHQ in an Ah-receptor-defective cells as well as in cells lacking a functional CYP1A1 protein (Prochaska and Talalay, 1988; Talalay, 1989; Friling *et al.*, 1990; Rushmore and Pickett, 1990). Whereas, bifunctional inducers such as β-napthoflavone (BNF) and 3-methylcholanthrene (3MC) can transcriptionally activate Phase II genes either directly through a Ah receptor-dependent mechanism, XRE/AhRE or they can also act through the ARE/EpRE once they are metabolized to compounds resembling monofunctional inducers. Non- or poorly-metabolizable inducers such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), transcriptionally activate Phase 2 genes only through the XRE/AhRE-Ah receptor-dependent mechanism (Prochaska and Talalay, 1988; Talalay, 1989; Friling *et al.*, 1990; Rushmore and Pickett, 1990). The interactions of these *cis*-acting elements (XRE/AhRE or ARE/EpRE) and respective functional *trans*-acting factors will initiate the gene expression of Phase II enzymes induced by these xenobiotics. Recently, several novel ARE-binding proteins have been identified, including the members of basic leucine zipper transcription factor (bZIP) family, Nrf1 (Venugopal and Jaiswal, 1996), Nrf2 (Venugopal and Jaiswal, 1996; Itoh *et al.*, 1997; Alam *et al.*, 1999; Moinova and Mulcahy, 1999), and Maf (Itoh *et al.*, 1997). A nuclear protein, designated as ARE-BP1, has also been described to constitutively bind to the ARE-inducible sequence, the GC box, and to be activated by tBHQ through a post-translational mecahnism (Wasserman and Fahl, 1997). Most recently, a cytosolic portein, named Keap1, has been identified to suppress Nrf2 transcriptional activity by retaining Nrf2 in the cytoplasm (Itoh *et al.*, 1999). However, currently, little is known about the upstream signal transduction events leading to the activation of these transcription factors in response to phenolic antioxidants and/or other monofunctional phase 2 gene inducers, leading to ARE-mediated phase 2 genes (e.g, QR, GST) expression.

Activation of mitogen activated protein kinase (MAPK): signal transduction events leading to gene expression

Mitogen-activated protein kinases (MAPKs), characterized as proline-directed serine/threonine [ProXSer/Thr-Pro; (Alvarez *et al.*, 1991; Gonzalez *et al.*, 1991)] kinases (Marshall, 1994), are important cellular signaling components which convert various extracellular signals into intracellular responses through serial phosphorylation cascades (Cobb and Goldsmith, 1995). At present time, three distinct but parallel MAP kinase cascades (ERK, JNK, and p38) have been identified in mammalian cells (Cano and Mahadevan, 1995; Kyriakis and Avruch, 1996) as shown in Fig 1. Each consists of a module of three kinases: a MAPK kinase kinase (MAPKKK), which phosphorylates and activates a MAPK kinase (MAPKK), which, in turn, phosphorylates and activates a MAPK. The best characterized MAPK pathway is a Ras-dependent activation of extracellular signal-regulated protein kinases (ERKs) in response to growth factors. In this pathway, tyrosine-phosphorylated transmembrane receptors associate with the SH2 domain of the adapter protein Grb2 (Skolnik *et al.*, 1993) and target nucleotide exchange factor SOS to the membrane-bound small G-protein Ras (Egan *et al.*, 1993). Activated Ras recruits Raf-1 (a MAPKKK) to the membrane, leading the activation of Raf-1 (Stokoe *et al.*, 1994). Once activated, Raf-1 can phosphorylate and activate a dual specificity kinase MEK (a MAPKK), which, in turn, activates ERK

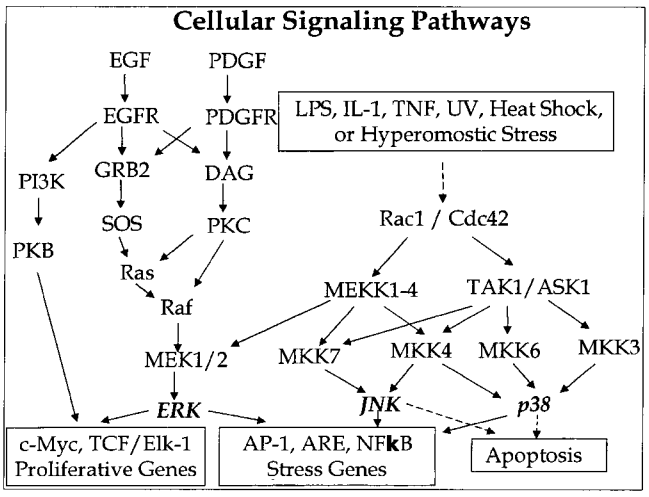

Fig. 1. Signal transduction events leading to the activation of the mitogen activated protein kinase (MAPK) pathway ERK, JNK, and p38, and the NFκB pathway, which culminates in gene expression, and potentially resulting in cell proliferative, survival, or apoptotic responses.

(a MAPK). In addition to tyrosine kinase receptors, certain G-protein-coupled receptors and PKC are also capable of activating ERK cascade (Burgering and Bos, 1995). Another emerging MAPK group is c-Jun N- terminal kinase (JNK), which is operated by a parallel signaling module, consisting of MEKK1/MKK4 (or SEK1, JNKK)/JNK (Hibi *et al.*, 1993; Kyriakis *et al.*, 1994). However, JNK cascade, unlike ERK pathway, is only modestly activated by growth factors and phorbol esters, and is instead strongly activated by stress signals such as UV light (Chen *et al.*, 1996b), g-radiation (Chen *et al.*, 1996a), protein synthesis inhibitors (Cano *et al.*, 1994), ceramide (Verheij *et al.*, 1996), DNA damaging agents (Yu *et al.*, 1996b), isothiocyanates chemopreventive agents (Yu *et al.*, 1996 a) and inflammatory cytokines (Raingeaud *et al.*, 1995). Therefore, JNK is also termed as stress-activated protein kinase (SAPK). A third parallel signaling module is called the p38 MAPK. p38 MAPK was originally identified in endotoxic lipopolysaccharide (LPS)-treated murine monocytes and macrophases as a homologue of a yeast gene, the high osmolarity glycerol response-1 (Hog1) (Brewster *et al.*, 1993; Han *et al.*, 1994). Independently, the p38 MAPK was identified in human cells as a cytokine-supppressive anti-inflammatory drug-binding protein (CSBP) (Lee *et al.*, 1994) or as a reactivating kinase (RK) (Freshney *et al.*, 1994; Rouse *et al.*, 1994). Recently, other new members of p38 subfamily have been cloned (Wang *et al.*, 1997). p38 is activated by phosphorylation on threonyl and tyrosyl residues within the tripeptide motif TGY by dual-specificity kinases, MKK3 and MKK6 (Derijard *et al.*, 1995) , which, in turn, are regulated by the upstream MAP kinase kinase kinases (MEKKs) and small GTP-binding proteins, Rac1 and Cdc42 (Kyriakis and Avruch, 1996). Once activated, these three MAPKs (ERK, JNK, and p38) can phosphorylate many transcription factors, such as c-Myc, p62TCF/Elk-1, c-Jun, ATF2, CHOP/GADD153, MEF2C, and SAP-1, and ultimately leading to the changes in gene expression (Karin, 1995; Karin, 1998). Given the fact that MAPKs are activated by such a wide range of factors, these signaling cascades may serve as a common mechanism and integrate with other signaling pathways to control cellular responses to various extracellular stimuli, including xenobiotics and pharmacological agents.

Cell death signaling

Cell death is the process which culminates with cessation of all biological activity. It is generally well accepted that apoptosis and necrosis are two distinct, mutually exclusive, modes of cell death (Darzynkiewicz *et al.*, 1997). Apoptosis, frequently referred to as programmed cell death, is an active and physiological mode of cell death, in which the cell itself designs and executes the program of its own demise and subsequent body disposal. While apoptosis is governed by an active participation of the affected cell, necrosis is passive, catabolic, and degenerative process. Necrosis generally represents a cells response to gross injury such as induction by an overdose of cytotoxic agents. The early event of necrosis is mitochondria swelling followed by rupture of the plasma membrane and release of cytoplasmic materials, which include proteolytic enzymes. Nuclear chromatin shows patchy areas of condensation, and the nucleus undergoes slow dissolution (karyolysis). Necrosis usually triggers an inflammatory reaction in the tissue and often resulting in scar formation. DNA degradation is not very extensive during necrosis as in the case of apoptosis, and the products of degraded DNA are heterogenous in size, failing to form discrete bands on electrophoretic gels.

Apoptosis or programmed cell death plays important roles in a variety of biological processes. For example, apoptosis plays a central role in both development and in homeostasis of multi-cellular eukaryotic organisms (Steller, 1995; Ashkenazi and Dixit, 1998). Cells die by apoptosis in the developing embryo during morphogenesis or synaptogenesis and in the adult animal during tissue repair / turnover or at the end of an immune response (Ashkenazi and Dixit, 1998). Because of the crucial physiological role of apoptosis, aberration of this process may have detrimental consequences. For instance, unscheduled apoptosis of certain brain neurons contributes to disorders such as Alzheimers and Parkinsons diseases, whereas the failure of dividing cells to initiate apoptosis after sustaining severe DNA damage may contribute to cancer (Thompson, 1995; Ashkenazi and Dixit, 1998).

Most growth factors prevent apoptosis, whereas death receptors such as tumor necrosis factor a (TNF-α) receptor, Fas (CD95 or Apo1), death receptor 3 (DR3; also called Apo3, WSL-1, TRAMP, or LARD), DR4, or DR5 (also called Apo2, TRAIL-R2, TRICK 2 or KILLER) (reviewed in (Ashkenazi and Dixit, 1998)) induce apoptosis. Death receptors are cell surface receptors that transmit apoptotic signals initiated on the plasma membrane by specific death ligands such as TNFa, FasL, or TRAIL (TNF related apoptosis induced ligand) play a central role in programmed cell death (Ashkenazi and Dixit, 1998). These death receptors belong to the TNF receptor gene superfamily, which contain a highly conserved cysteine-rich extracellular domains (Smith *et al.*, 1994). In addition, the death receptors also contain a highly conserved cytoplasmic death domain (Tartaglia *et al.*, 1993; Nagata, 1997). The death domains enable the death receptors to transmit signal from outside the cell to the inside of the cell via interaction with the intracellular apoptotic machinery such as the ICE/Ced-3 family proteases (caspases). To date, there are at least 15 different members of caspases in mammalian cells.

These include ICE, CPP32, ICH-1, -2, MCH-2, -3, -4, -5, -6, and TY) reviewed in (Thornberry and Lazebnik, 1998); (Fernandes-Alnemri *et al.*, 1994; Tewari *et al.*, 1995; Alnemri *et al.*, 1996; Chinnaiyan *et al.*, 1997). These caspases are synthesized as precursors that are normally inactive. Activation involves processing of a prodomain located at the amino terminus of the protein coupled with cleavages which generate two subunits of approximately 20 and 10 kDa. The activated form of these enzymes are found to be tetramer composed of two 20 and two 10 kDa subunits. Although overexpression of many of these caspases in many cells leads to apoptosis, CPP32 (caspase 3) is thought to play a more general role in mediating apoptosis induced by various stimuli. The exact physiological substrates of CPP32 is currently unknown, but may involve β-actin and poly-(ADP-ribose) polymerase or PARP (Martin and Green, 1995). Proteolytic cleavage of these proteins may result in the apoptotic morphological changes characterized by a decrease in volume and buoyant density, chromatin condensation, DNA fragmentation, and cell surface blebbing (McGowan *et al.*, 1996; Liu *et al.*, 1997). Thus, engagement of the membrane death receptors with the appropriate death ligands can activate death caspases within seconds of ligand binding (shown in Fig. 2), causing an apoptotic demise of the cell within hours (Ashkenazi and Dixit, 1998).

In addition to these physiological regulators of apoptosis via the death ligands and death receptors complexation, many environmental stresses also cause apoptosis. Recent studies have suggested that oxidative stress may play a critical role in apoptosis through these signals (Buttke and Sandstrom, 1994; Busciglio and Yankner, 1995; Briehl, 1996; Slater, 1996). The mitochondria may orchestrate the process of apoptosis (Fig. 2), and these have been established in many systems (reviewed in (Green and Reed, 1998)). During apoptosis (*in vitro* and *in vivo*) cytochrome c (Cyto c) is released from the mitochondria, and this is inhibited by the presence of the anti-apoptotic protein Bcl-2 (Kluck *et al.*, 1997; Yang *et al.*, 1997). Cytosolic cytochrome c may form a critical part of the vertebrate apoptosome, which comprises cytochrome c, Apaf-1 (apoptotic protease activating factor 1) and procaspase 9 (Li *et al.*, 1997). The consequence of this complexation is the activation of caspase 9, which then processes and activates other caspases (upstream or downstream) to coordinate the biochemical execution of apoptotic cell death (Green and Reed, 1998) (Fig. 2).

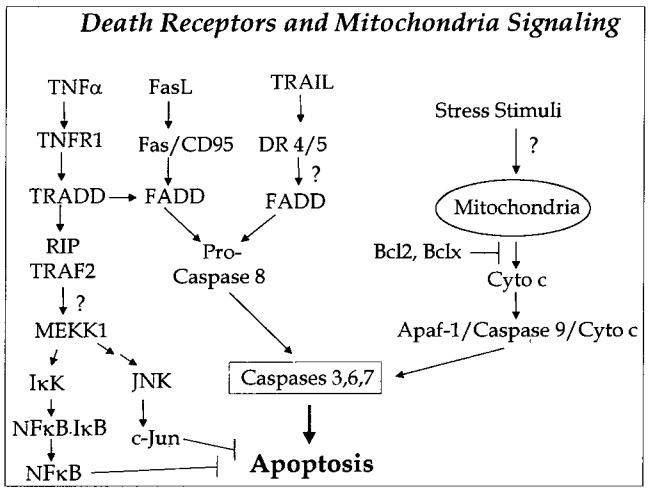

Fig. 2. Signal transduction events of cell death pathway triggered by either cell surface membrane death receptors, or mitochondrial pathway which may result in the blockade of apoptosis in some instances, but generally resulting in apoptosis.

Illustrative examples: phenolic compounds activate MAPK pathway and induce gene expression a cell survival response

Phenolic antioxidant BHA has been shown to be potent chemopreventive agent (Wattenberg, 1973; Benson *et al.*, 1978; Wattenberg *et al.*, 1980; King and McCay, 1983; Wattenberg, 1983; Wattenberg, 1985; Talalay, 1987) and a potent Phase II gene inducer (Benson *et al.*, 1978; Lam *et al.*, 1980; Sparnins *et al.*, 1982a; Sparnins *et al.*, 1982b). Recent studies from our laboratory showed that BHA potently activated JNK1, and ERK2 activities in many mammalian cell lines such as human cervical squamous carcinoma HeLa cells, human hepatoma HepG2 cells and murine hepatoma Hepa1c1c7 cells (Yu *et al.*, 1997b) in a time- and concentration-dependent fashion. Similarly, tBHQ, the demethylated active metabolite of BHA, which is also a potent inducer of Phase II genes (De Long *et al.*, 1985; Friling *et al.*, 1990; Rushmore *et al.*, 1991), stimulated the activity of ERK2, but not JNK, in Hepa1c1c7 and HepG2 cells (Yu *et al.*, 1997b; Yu *et al.*, 1999). Exposure of human hepatoma HepG2 cells to 10 to 100 mg/ml of green tea polyphenols (GTP) activated JNK1 activity (Yu *et al.*, 1997a). Activation of JNK activity was seen at 30 min, peaked at 120 min, and sustained up to 6 h, whereas, activation of ERK2 activity occurred only at higher concentrations (250 mg/ml to 1 mg/ml), with much earlier kinetics (detected at 5 min, peaked at 15 min, and declined after 120 min) (Yu *et al.*, 1997a). These data suggest that activation of ERK2 and JNK1 activities by GTP may occur by differential mechanisms. One of the consequences of the activation of MAPK by extracellular stimuli including GTP would implicate the induction of gene expression. Indeed, GTP induced the mRNA expression of immediate early genes such as *c-jun*, and *c-fos*, as well as

transcriptionally activated the ARE/EpRE chloramphenicol acetyltransferase (CAT) reporter gene, which is present in many stress-response genes identifiable in the GeneBank (Wasserman and Fahl, 1997) including many Phase II drug metabolizing enzymes, GST, QR, (Rushmore and Pickett, 1990; Li and Jaiswal, 1992; Prestera *et al.*, 1993) and genes encoding for the transcriptional factor such as hepatocyte nuclear factor 3/fork head homolog 11(Ye *et al.*, 1997).

Studies with BHA in HeLa cells demonstrated rapid but transient activation of ERK2 activity whereas a more delayed and sustained JNK1 activity, similar to that induced by GTP as described above (Yu *et al.*, 1997b). In contrast, its active metabolite tBHQ activated ERK2, but weakly stimulated JNK1 activity. Furthermore, activation of ERK2 by tBHQ was delayed and prolonged, showing kinetics different from that stimulated by BHA. This difference in activation kinetics suggests that the signaling events elicited by BHA may not be dependent on its metabolite tBHQ *via* metabolic demethylation. ERK2 activation by BHA and tBHQ required the involvement of an upstream signaling kinase MEK, as evidenced by the inhibitory effect of a MEK inhibitor, PD98059 (Yu *et al.*, 1997b). Pretreatment with *N*-acetyl-L-cysteine (NAC), glutathione (GSH), and vitamin E, inhibited ERK2 activation by BHA and tBHQ, but not JNK1 activity. These data again suggest that differential mechanisms may be involved in the activation of ERK2 and JNK1 activities by BHA and tBHQ. The activation of ERK2 by BHA and tBHQ may involve phenoxyl radicals and/or their derivatives (Yu *et al.*, 1997b), whereas activation of JNK1 may involve intracellular calcium and protein kinase C (PKC) (Yu, 1998b).

To elucidate the physiological role of MAPK in the regulation of Phase II gene expression, recently we have reported that inhibition of ERK pathway with its inhibitor, PD98059, abolished EKR2 activation and impaired the induction of QR enzymatic activity and ARE-luciferase (LUC) reporter gene by tBHQ (Yu *et al.*, 1999). Overexpression of a dominant-negative mutant of ERK2 also attenuated tBHQ induction of ARE-LUC reporter gene activity (Yu *et al.*, 1999). This result suggests that ERK2 plays a positive role in the induction of ARE-LUC and Phase II QR enzyme by phenolic antioxidant such as tBHQ. Since, tBHQ does not stimulate JNK1 activity, this pathway may not contribute to the induction of ARE-LUC and phase 2 NQO1 enzyme by tBHQ. In contrast, BHA potently stimulated JNK1 activity, the role of JNK1 in the induction of ARE-LUC and Phase II QR enzyme by BHA is currently under investigation.

Most recently, we have found that both BHA and tBHQ stimulated the third MAPK, p38 in a time- and dose-dependent fashion in Hepa1c1c7 and HepG2 cells (Yu, February 2000 (in the press)). Inhibition of p38 activation by its inhibitor, SB203580, enhanced the induction of QR activity and the activation of ARE-LUC reporter gene by tBHQ. In contrast, SB202474, an inactive structural analog of SB203580, had little effect. Consistent with this result, interfering with p38 kinase pathway by overexpression of a dominant-negative mutant of p38 or MKK3, an immediate upstream regulator of p38, potentiated the activation of ARE-LUC reporter gene by tBHQ, whereas the wild type of p38 and MKK3 diminished such activation. In addition, inhibition of p38 activity augmented the induction of ARE-LUC reporter gene activity by BHA, and b-naphthoflavone (BNF). Thus, p38 MAPK pathway may function as a negative regulator in the ARE-mediated induction of Phase II QR enzyme. These results suggest that the coordinate modulation of MAPK cascades may be critical in the regulation of Phase II genes through the ARE/EpRE induced by various xenobiotics.

Recent studies from several laboratories showed that the basic-leucine zipper (bZIP) transcription factors, including Nrf1 (Venugopal and Jaiswal, 1996), Nrf2 (Venugopal and Jaiswal, 1996; Itoh *et al.*, 1997) and small Maf (Itoh *et al.*, 1997) were implicated in the binding and gene activation through ARE sequences from rat and human gene promoters. The importance of Nrf2 in the transcriptional activation of ARE-reporter genes has been confirmed recently in other ARE-mediated genes including human g-glutamylcysteine synthetase (GCS) (Moinova and Mulcahy, 1999), and mouse heme oxygenase-1 (HO-1) (Alam *et al.*, 1999). In knockout studies, the induction of QR and GST by BHA was largely eliminated in the liver and intestine of Nrf2-/- mice (Itoh *et al.*, 1997), and the gene expression of several detoxification enzymes including QR is markedly reduced in the lung of Nrf2-/- mice (Chan and Kan, 1999). This lack of Phase II enzyme induction in Nrf2-/- mice suggests that Nrf2 is the most likely transcriptional factor involved in the transcriptional activation of ARE-mediated phase 2 gene induction. Recent data from our laboratory found that co-transfection of different amount of Nrf2 expression plasmid (Moi *et al.*, 1994) with ARE-LUC reporter construct, the transcriptional activation of ARE-LUC reporter gene was increased in a dose-dependent manner as compared to the control vector (unpublished observations). Dominant negative (DN) mutant (deletion of N-terminal 1-430 amino acid residues) of Nrf2 blocked transcriptional activation of ARE-LUC reporter gene, as well as blocked transcriptional induction of ARE-LUC by inducers of Phase II genes including BHA and tBHQ. Other member of the bZIP transcription factor such as c-Jun which binds AP-1 DNA element, are transcriptionally activated by the MAPK pathway (Karin, 1998). Recently, we have reported that Ras and c-Raf (the upstream regulators of ERK MAPK; as shown in Fig. 1) are involved in the induction of ARE-dependent Phase II QR gene (Yu *et*

al., 1999). We have conducted preliminary studies to determine whether Ras and c-Raf can enhance transcriptional activity of Nrf2 using ARE-LUC reporter gene. We found that the activated forms of Ras(61L) and c-Raf(BXB) substantially enhanced Nrf2 transcriptional activity of ARE-LUC reporter gene; whereas, their dominant negative mutants had no effect (unpublished observations). Taken together, our observations suggest that stimulation of MAPK by phenolic antioxidants BHA/tBHQ may play a critical role in the early signaling events leading to transcriptional activation of Nrf2 transcription factor and subsequent coupling to the ARE/EpRE DNA element, presents in many stress response genes including Phase II drug metabolizing enzymes.

Illustrative examples: isothiocyantes activate mapk pathway and induce gene expression a cell survival response

Similar to the phenolic compounds described above, exposure of cells to various natural or synthetic chemopreventive isothiocyanates such as phenethyl isothiocyanate (PEITC), and sulfarophane, induce expression of Phase II drug metabolizing enzymes such as GST, QR, and UGT (Sparnins *et al.*, 1982a; Guo *et al.*, 1992; Prestera *et al.*, 1993; Fei *et al.*, 1996). We and other investigators have recently shown that various isothiocyanates including PEITC (Yu *et al.*, 1996a), sulfarophane (Yu *et al.*, 1999), and phenylmethyl isothiocyanate (PMITC) or benzyl isothiocyanate (Patten and DeLong, 1999) activated the MAPK pathway. However, differences exist in the activation of MAPK by PEITC and sulforaphane in HepG2 cells. For example, PEITC stimulated both JNK and ERK activities, whereas sulforaphane stimulated only the ERK activity but inhibited both basal and UV-stimulated JNK activity (Yu *et al.*, 1999), suggesting that the difference in chemical structure may play an important determinant in the activation of MAPK by the different isothiocyanates. This may be due to the presence of the aromatic ring in PEITC which is more reactive (Michael acceptor) versus the sulfone for sulforaphane (Zhang *et al.*, 1992; Zhang *et al.*, 1995; Jiao *et al.*, 1997), or differences in lipophilicity between the two compounds. Future studies will elucidate their mechanistic differences. Similar to tBHQ described above, inhibition of MAPK kinase with its inhibitor, PD98059, abolished ERK2 activation and impaired the induction of QR enzyme activity, and ARE-reporter gene activity by sulforaphane (Yu et *al.*, 1999). Overexpression of a dominant negative mutant of ERK2 also attenuated sulforaphane induction of ARE-reporter gene activity. Interestingly, expression of Ras and its constitutively activated form of Ras (61 L) showed increased basal ARE-reporter gene activity, they did not affect the activation of reporter gene by the inducers sulforaphane or tBHQ (Yu *et al.*, 1999). Furthermore, a dominant-negative mutant of Ras had little effect on ERK2 activation by sulforaphane or tBHQ, implicating a Ras-independent mechanism. Indeed, both sulforaphane and tBHQ were able to stimulate Raf-1 kinase activity in vivo as well as in vitro (Yu *et al.*, 1999). Thus, our results indicate that the induction of ARE-dependent phase II detoxifying enzymes may be mediated by a MAPK pathway, which may involve direct activation of the Raf-1 kinase by the isothiocyanates (sulforaphane) and phenolic compounds (BHA and tBHQ).

Illustrative examples: phenolic compounds and isothiocyantes activate ice/ced-3 proteases (caspases) leading to apoptosis

Naturally occurring chemopreventive agents that induce phase 2 detoxifying enzymes such as GST, and QR as discussed above, can prevent cancer by enhancing elimination of endogenous and/or exogenous chemical carcinogens. On the other hand, the induction of apoptosis or programmed cell death of precancerous cells by these compounds may provide a distinct mechanism for their chemopreventive functions (Pascale *et al.*, 1992; Delia *et al.*, 1993; Fesus *et al.*, 1995; 1996; Kelloff *et al.*, 1996; Lotan, 1996; Lupulescu, 1996; Yu, 1998a). The phenolic compounds such as quercetin (Castillo *et al.*, 1989), apigenin (Hirano *et al.*, 1989), green tea extracts (Yan, 1990) have been shown to be antitumor, or antiproliferative on many cancer cell lines. Recently many studies have clearly demonstrated that various chemopreventive agents both natural and synthetic can trigger apoptosis of many human cancer types (Ahmad *et al.*, 1997; Samaha *et al.*, 1997; Weinstein *et al.*, 1997; Yu *et al.*, 1997b; Langman and Boyle, 1998; Shao *et al.*, 1998; Yang *et al.*, 1998; Yu, 1998a; Crowell, 1999; Kirlin *et al.*, 1999; Lin *et al.*, 1999).

To understand the signaling events leading to apoptosis elicited by chemicals or natural products, we have studied the concentration- or dose-response of various compounds. In human hepatoma HepG2 cells, low concentrations of BHA (50 to 100 μM) activated ERK2 and JNK1, whereas tBHQ activated only ERK2 (Yu *et al.*, 1997b), as discussed above. At these concentrations, the survival of HepG2 cells was not affected by either BHA or tBHQ, but at concentrations above 100 μM, both BHA and tBHQ began to induce cell growth inhibitory effect (Yu *et al.*, 1997b). These results were similar to that previously reported by Choi et al (Choi and Moore, 1993) in HepG2 cells, low concentrations (100 μM) of BHA, it induced *c-jun* and *c-fos* mRNA expression, and reached maximum induction at 150 μM in HepG2 cells, whereas cytotoxic effects were seen at BHA concentrations above 150 mM. Our recent studies with various green tea polyphenols, such as (-)-epigal-

locatechin-3-gallate (EGCG), and epicatechin gallate (ECG) strongly activated MAPK (ERK, JNK and p38), as well as induction of ARE-reporter gene activity between 100 to 250 μM concentrations, whereas potent cytotoxicity effects were observed at 500 μM (unpublished observations).

Previously, it was not clear how these phenolic antioxidants, such as BHA and tBHQ, induce cell death at higher concentrations, which may be associated with the toxicity observed in animals (Mizutani *et al.*, 1987; Clayson *et al.*, 1990). To address this cytotoxicity issue, we have studied the activation of the cell death protein caspases, particularly caspase-1 (ICE or ICE-like) and caspase-3 (CPP-32 or CPP-32-like) families, induced by BHA and tBHQ. Treatment of HeLa cells with 250 to 500 μM of BHA led to the activation of caspase-3 activity in a time- and concentration-dependent fashion (Yu, 1998b). Nuclei staining of the cells with diamidino-2-phenylindole (DAPI) as described previously (Lei *et al.*, 1998; Yu, 1998a), apoptotic morphologies such as chromatin condensation, and cell surface membrane blebbing were observed, indicating that most of the cells died *via* apoptosis. When BHA concentrations increased above 750 mM, extensive necrosis occurred very rapidly within 2 hr, as shown by trypan blue staining (Yu, 1998b). Most recently, we have found that BHA-induced apoptosis in primary cultured of rat hepatocytes triggered mitochondria permeability transition (PT) within 30 min of treatment and was inhibited by the pretreatment with cyclosporin A, a ligand of cyclophilin D, which prevents mitochondrial PT. This was followed by cytochrome c release and activation of caspases such as caspases 9, 3, and 8, but not caspase 1 (unpublished observations). Future *in vivo* animal studies will elucidate whether this concentration-dependent activation of MAPK, caspases, and apoptosis induced by phenolic compounds such as BHA or EGCG will occur in normal, preneoplastic, and/or tumor tissues.

The effects of various isothiocyanates on the activation of MAPK (Yu *et al.*, 1996a) and caspase (Yu, 1998a) pathways in various mammalian cells including HeLa cells have been performed in our laboratory. Treatment of HeLa cells with 5 - 10 μM of PEITC (in phenol red-free medium) strongly activated JNK1 in a time- and concentration-dependent manner. Previous studies that were conducted in phenol-red medium, necessitated the use of higher concentrations of PEITC to activate JNK activity (Yu *et al.*, 1996a). PEITC stimulated both JNK1 and ERK2 activities in HepG2 cells at similar concentrations. Increasing the concentrations of PEITC from 10 to 20 μM, strongly activated caspase-3-like (CPP32-like) protease activity in HeLa cells, and induced apoptosis as demonstrated by DAPI nuclear staining (Yu, 1998a). Pretreatment of cells with a specific inhibitor of caspase-3-like proteases, Ac-DEVD-CHO, attenuated PEITC-induced caspase-3 activity, and apoptosis. Other structurally related isothiocyanates, PMITC, phenylbutyl isothiocyanate (PBITC) and phenylhexyl isothiocyanate (PHITC) but not phenyl isothiocyanate (PITC) induced apoptosis in HeLa cells in a time- and concentration-dependent manner. Similar observation with PMITC was recently reported in human colon carcinoma (HT29) cell line (Kirlin *et al.*, 1999). These isothiocyanates, except PITC, also stimulated proteolytic activity of caspase-3, leading to the cleavage of a protein substrate PARP (Yu, 1998a). In contrast, caspase-1 activity was not stimulated by these compounds. Further increase in PEITC concentrations induced cell death predominantly *via* necrosis. These results suggest that compounds such as PEITC has a narrower therapeutic window than phenolic antioxidants BHA or tBHQ as described above, in the various human and rodent cancer cell lines tested. Future studies will determine whether these concentration-dependent effects will be observed *in vivo* in animal studies.

DISCUSSION

In summary, our studies with natural products including flavonoids (GTP, EGCG, ECG), and various structurally related isothiocyanates (e.g., PMITC, PEITC, and sulforaphane), as well as studies with different chemicals such as phenolic antioxidants (BHA, tBHQ), have provided important insights into the signal transduction pathways induced by these compounds. At low concentrations, these chemicals activate the MAPKs pathway (ERK2, JNK1, and/or p38) which may lead to the induction of gene expression such as c-Fos, c-Jun, GST, and QR, resulting in protection and/or survival mechanisms (Fig. 3). Increasing the concentrations of the compounds will also activate the MAPK pathway, however, the ICE/Ced-3 protease (caspase) pathway will also be activated concomitantly, which will lead to apoptotic cell death. Further increment of concentrations to suprapharmacological concentrations will lead to nonspecific cell death predominantly occurring *via* necrosis. This concentration-dependent cellular response phenomenon may explain in part the beneficial pharmacological effects observed in animals after administration of low doses of BHA, whereas its undesirable toxicological responses after very high doses (Mizutani *et al.*, 1987; Clayson *et al.*, 1990). Furthermore, the wide and narrow concentration ranges between the activation of MAPK/gene induction and caspases/cell death by phenolic compounds and isothiocyanates in mammalian cell cultures, may reflect the wide and narrow therapeutic windows exhibited by these agents *in vivo*. Studying of cellular signal transduction events elicited by various natural products would yield important insights into the regulation of gene expression which will potentially resulting in homeostatic cell

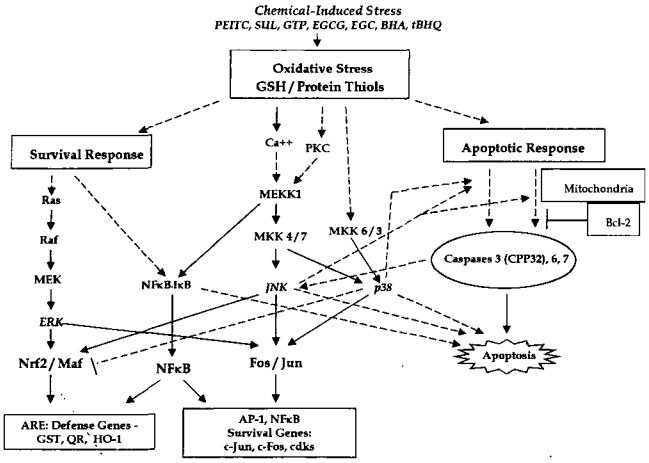

Fig. 3. Schematic representation of xenobiotic-induced oxidative stress leading to activation of the MAPK pathway ERK, JNK, or p38, and the NFκB pathway, which results in gene expression, and potentially cell survival response, or the activation of the ICE/Ced-3 (caspase) pathway leading to cell death and apoptotic response.

survival response, and activation of cell death proteins which will potentially resulting in beneficial effects if occurred in preneoplastic or tumor cells, however, may result in cytotoxicity if occurred in normal cells. Consequently, the studies of these and other signaling pathways will advance our knowledge and understanding of the efficacy and safety of many natural products of which many may become important therapeutic drugs of the future.

ACKNOWLEDGEMENTS

We thank Drs. David Ucker and Tse-Hua Tan for the exciting collaborative efforts and our colleagues for helpful discussions. This work was supported by the National Institutes of Health grant R01-CA73674 to A.-N. T. Kong.

REFERENCES

NSAIDs may prevent colon cancer through apoptosis, not anti- inflammatory effects [news]. *Oncology,* 10, 624 (1996).

Prevention of cancer in the next millenium: Report of the chemoprevention working group to the American Association for Cancer Research. *Cancer Research,* 59, 4743-4758 (1999).

Ahmad, N., Feyes, D. K., Nieminen, A. L., Agarwal, R. and Mukhtar, H., Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *J Natl Cancer Inst,* 89, 1881-1886 (1997).

Alam, J., Stewart, D., Touchard, C., Boinapally, S., Choi, A. M. and Cook, J. L., Nrf2, a Cap'n'Collar transcription factor, regulates induction of the heme oxygenase-1 gene. *J Biol Chem,* 274, 26071-26078 (1999).

Alnemri, E. S., Livingston, D. J., Nicholson, D. W., Salvesen, G., Thornberry, N. A., Wong, W. W. and Yuan, J., Human ICE/CED-3 protease nomenclature [letter]. *Cell,* 87, 171 (1996).

Alvarez, E., Northwood, I. C., Gonzalez, F. A., Latour, D. A., Seth, A., Abate, C., Curran, T. and Davis, R. J., Pro-Leu-Ser/Thr-Pro is a consensus primary sequence for substrate protein phosphorylation. Characterization of the phosphorylation of c- myc and c-jun proteins by an epidermal growth factor receptor threonine 669 protein kinase. *J Biol Chem,* 266, 15277-15285 (1991).

Ashkenazi, A. and Dixit, V. M., Death receptors: signaling and modulation. *Science,* 281, 1305-1308 (1998).

Batzinger, R. P., Ou, S. Y. and Bueding, E., Antimutagenic effects of 2(3)-tert-butyl-4-hydroxyanisole and of antimicrobial agents. *Cancer Res,* 38, 4478-4485 (1978).

Benson, A. M., Barretto, P. B. and Stanley, J. S., Induction of DT-diaphorase by anticarcinogenic sulfur compounds in mice. *J Natl Cancer Inst,* 76, 467-473 (1986).

Benson, A. M., Batzinger, R. P., Ou, S. Y., Bueding, E., Cha, Y. N. and Talalay, P., Elevation of hepatic glutathione S-transferase activities and protection against mutagenic metabolites of benzo(a)pyrene by dietary antioxidants. *Cancer Res,* 38, 4486-4495 (1978).

Bogaards, J. J., van Ommen, B., Falke, H. E., Willems, M. I. and van Bladeren, P. J., Glutathione S-transferase subunit induction patterns of Brussels sprouts, allyl isothiocyanate and goitrin in rat liver and small intestinal mucosa: a new approach for the identification of inducing xenobiotics. *Food Chem Toxicol,* 28, 81-88 (1990).

Brewster, J. L., de Valoir, T., Dwyer, N. D., Winter, E. and Gustin, M. C., An osmosensing signal transduction pathway in yeast. *Science,* 259, 1760-1763 (1993).

Briehl, M. M., and Baker, A.F., Modulation of the antioxidant defense as a factor in apoptosis. *Cell Death and Differentiation,* 3, 63-70 (1996).

Buetler, T. M., Gallagher, E. P., Wang, C., Stahl, D. L., Hayes, J. D. and Eaton, D. L., Induction of phase I and phase II drug-metabolizing enzyme mRNA, protein, and activity by BHA, ethoxyquin, and oltipraz. *Toxicol Appl Pharmacol,* 135, 45-57 (1995).

Burgering, B. M. and Bos, J. L., Regulation of Ras-mediated signalling: more than one way to skin a cat. *Trends Biochem Sci,* 20, 18-22 (1995).

Busciglio, J. and Yankner, B. A., Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro. *Nature,* 378, 776-779 (1995).

Buttke, T. M. and Sandstrom, P. A., Oxidative stress as a

mediator of apoptosis [see comments]. *Immunol Today,* 15, 7-10 (1994).

Cano, E., Hazzalin, C. A. and Mahadevan, L. C., Anisomycin-activated protein kinases p45 and p55 but not mitogen-activated protein kinases ERK-1 and -2 are implicated in the induction of c-fos and c-jun. *Mol Cell Biol,* 14, 7352-7362 (1994).

Cano, E. and Mahadevan, L. C., Parallel signal processing among mammalian MAPKs. *Trends Biochem Sci,* 20, 117-122 (1995).

Castillo, M. H., Perkins, E., Campbell, J. H., Doerr, R., Hassett, J. M., Kandaswami, C. and Middleton, E., Jr., The effects of the bioflavonoid quercetin on squamous cell carcinoma of head and neck origin. *Am J Surg,* 158, 351-355 (1989).

Cha, Y. N. and Bueding, E., Effects of 2(3)-tert-butyl-4-hydroxyanisole administration on the activities of several hepatic microsomal and cytoplasmic enzymes in mice. *Biochem Pharmacol,* 28, 1917-1921 (1979).

Cha, Y. N. and Heine, H. S., Comparative effects of dietary administration of 2(3)-tert-butyl-4-hydroxyanisole and 3,5-di-tert-butyl-4-hydroxytoluene on several hepatic enzyme activities in mice and rats. *Cancer Res,* 42, 2609-2615 (1982).

Chan, K. and Kan, Y. W., Nrf2 is essential for protection against acute pulmonary injury in mice [In Process Citation]. *Proc Natl Acad Sci U S A,* 96, 12731-12736 [Record as supplied by publisher] (1999).

Chen, Y. R., Meyer, C. F. and Tan, T. H., Persistent activation of c-Jun N-terminal kinase 1 (JNK1) in gamma radiation-induced apoptosis. *J Biol Chem,* 271, 631-634 (1996a).

Chen, Y. R., Wang, X., Templeton, D., Davis, R. J. and Tan, T. H., The role of c-Jun N-terminal kinase (JNK) in apoptosis induced by ultraviolet C and gamma radiation. Duration of JNK activation may determine cell death and proliferation. *J Biol Chem,* 271, 31929-31936 (1996b).

Chinnaiyan, A. M., K, O. R., Lane, B. R. and Dixit, V. M., Interaction of CED-4 with CED-3 and CED-9: a molecular framework for cell death [see comments]. *Science,* 275, 1122-1126 (1997).

Choi, H. S. and Moore, D. D., Induction of c-fos and c-jun gene expression by phenolic antioxidants. *Mol Endocrinol,* 7, 1596-1602 (1993).

Clayson, D. B., Iverson, F., Nera, E. A. and Lok, E., The significance of induced forestomach tumors. *Annu Rev Pharmacol Toxicol,* 30, 441-463 (1990).

Cobb, M. H. and Goldsmith, E. J., How MAP kinases are regulated. *J Biol Chem,* 270, 14843-14846 (1995).

Conney, A. H., Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. *Cancer Res,* 42, 4875-4917 (1982).

Crowell, P. L., Prevention and therapy of cancer by dietary monoterpenes. *J Nutr,* 129, 775S-778S (1999).

Darzynkiewicz, Z., Juan, G., Li, X., Gorczyca, W., Murakami, T. and Traganos, F., Cytometry in cell necrobiology: analysis of apoptosis and accidental cell death (necrosis). *Cytometry,* 27, 1-20 (1997).

De Long, M. J., Prochaska, H. J. and Talalay, P., Tissue-specific induction patterns of cancer-protective enzymes in mice by tert-butyl-4-hydroxyanisole and related substituted phenols. *Cancer Res,* 45, 546-551 (1985).

Delia, D., Aiello, A., Lombardi, L., Pelicci, P. G., Grignani, F., Formelli, F., Menard, S., Costa, A., Veronesi, U. and *et al.*, N-(4-hydroxyphenyl)retinamide induces apoptosis of malignant hemopoietic cell lines including those unresponsive to retinoic acid. *Cancer Res,* 53, 6036-6041 (1993).

Derijard, B., Raingeaud, J., Barrett, T., Wu, I. H., Han, J., Ulevitch, R. J. and Davis, R. J., Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms [published erratum appears in Science 1995 Jul 7;269(5220):17]. *Science,* 267, 682-685 (1995).

Egan, S. E., Giddings, B. W., Brooks, M. W., Buday, L., Sizeland, A. M. and Weinberg, R. A., Association of Sos Ras exchange protein with Grb2 is implicated in tyrosine kinase signal transduction and transformation [see comments]. *Nature,* 363, 45-51 (1993).

Favreau, L. V. and Pickett, C. B., Transcriptional regulation of the rat NAD(P)H:quinone reductase gene. Identification of regulatory elements controlling basal level expression and inducible expression by planar aromatic compounds and phenolic antioxidants. *J Biol Chem,* 266, 4556-4561 (1991).

Fei, P., Matwyshyn, G. A., Rushmore, T. H. and Kong, A. N. T., Transcription regulation of rat glutathione S-transferase Ya subunit gene expression by chemopreventive agents. *Pharm Res,* 13, 1043-1048 (1996).

Fernandes-Alnemri, T., Litwack, G. and Alnemri, E. S., CPP32, a novel human apoptotic protein with homology to Caenorhabditis elegans cell death protein Ced-3 and mammalian interleukin-1 beta-converting enzyme. *J Biol Chem,* 269, 30761-30764 (1994).

Fesus, L., Szondy, Z. and Uray, I., Probing the molecular program of apoptosis by cancer chemopreventive agents. *J Cell Biochem Suppl,* 22, 151-161 (1995).

Freshney, N. W., Rawlinson, L., Guesdon, F., Jones, E., Cowley, S., Hsuan, J. and Saklatvala, J., Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of Hsp27. *Cell,* 78, 1039-1049 (1994).

Friling, R. S., Bensimon, A., Tichauer, Y. and Daniel, V., Xenobiotic-inducible expression of murine glutathione S-transferase Ya subunit gene is controlled by an electrophile-responsive element. *Proc Natl Acad Sci U S A,* 87, 6258-6262 (1990).

Fujita, Y., Yamane, T., Tanaka, M., Kuwata, K., Okuzumi,

J., Takahashi, T., Fujiki, H. and Okuda, T., Inhibitory effect of (-)-epigallocatechin gallate on carcinogenesis with N-ethyl-N'-nitro-N-nitrosoguanidine in mouse duodenum. *Jpn J Cancer Res,* 80, 503-505 (1989).

Gerhauser, C., You, M., Liu, J., Moriarty, R. M., Hawthorne, M., Mehta, R. G., Moon, R. C. and Pezzuto, J. M., Cancer chemopreventive potential of sulforamate, a novel analogue of sulforaphane that induces phase 2 drug-metabolizing enzymes. *Cancer Res,* 57, 272-278 (1997).

Glatt, H., Sulfation and sulfotransferases 4: bioactivation of mutagens via sulfation. *Faseb J,* 11, 314-321 (1997).

Gonzalez, F. A., Raden, D. L. and Davis, R. J., Identification of substrate recognition determinants for human ERK1 and ERK2 protein kinases. *J Biol Chem,* 266, 22159-22163 (1991).

Green, D. R. and Reed, J. C., Mitochondria and apoptosis. *Science,* 281, 1309-1312 (1998).

Guenthner, T., Epoxide Hydrolase. In Mulder, G., (Eds). *Conjugation Reactions in Drug Metabolism*. Taylor & Francis, London, pp. 365-404, 1990.

Guo, Z., Smith, T. J., Wang, E., Eklind, K. I., Chung, F. L. and Yang, C. S., Structure-activity relationships of arylalkyl isothiocyanates for the inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone metabolism and the modulation of xenobiotic-metabolizing enzymes in rats and mice. *Carcinogenesis,* 14, 1167-1173 (1993).

Guo, Z., Smith, T. J., Wang, E., Sadrieh, N., Ma, Q., Thomas, P. E. and Yang, C. S., Effects of phenethyl isothiocyanate, a carcinogenesis inhibitor, on xenobiotic-metabolizing enzymes and nitrosamine metabolism in rats. *Carcinogenesis,* 13, 2205-2210 (1992).

Han, J., Lee, J. D., Bibbs, L. and Ulevitch, R. J., A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science,* 265, 808-811 (1994).

Hecht, S. S., Chemoprevention by isothiocyanates. *J Cell Biochem Suppl,* 22, 195-209 (1995).

Hengstler, J. G., Arand, M., Herrero, M. E. and Oesch, F., Polymorphisms of N-acetyltransferases, glutathione S-transferases, microsomal epoxide hydrolase and sulfotransferases: influence on cancer susceptibility. *Recent Results Cancer Res,* 154, 47-85 (1998).

Hibi, M., Lin, A., Smeal, T., Minden, A. and Karin, M., Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev,* 7, 2135-2148 (1993).

Hirano, T., Oka, K. and Akiba, M., Antiproliferative effects of synthetic and naturally occurring flavonoids on tumor cells of the human breast carcinoma cell line, ZR- 75-1. *Res Commun Chem Pathol Pharmacol,* 64, 69-78 (1989).

Igarashi, K., Kataoka, K., Itoh, K., Hayashi, N., Nishizawa, M. and Yamamoto, M., Regulation of transcription by dimerization of erythroid factor NF-E2 p45 with small Maf proteins [see comments]. *Nature,* 367, 568-572 (1994).

Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Oyake, T., Hayashi, N., Satoh, K., Hatayama, I., Yamamoto, M. and Nabeshima, Y., An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun,* 236, 313-322 (1997).

Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J. D. and Yamamoto, M., Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev,* 13, 76-86 (1999).

Jaiswal, A. K., Jun and Fos regulation of NAD(P)H: quinone oxidoreductase gene expression. *Pharmacogenetics,* 4, 1-10 (1994).

Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W., Fong, H. H., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G., Moon, R. C. and Pezzuto, J. M., Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science,* 275, 218-220 (1997).

Jiao, D., Smith, T. J., Yang, C. S., Pittman, B., Desai, D., Amin, S. and Chung, F. L., Chemopreventive activity of thiol conjugates of isothiocyanates for lung tumorigenesis. *Carcinogenesis,* 18, 2143-2147 (1997).

Karin, M., The regulation of AP-1 activity by mitogen-activated protein kinases. *J Biol Chem,* 270, 16483-16486 (1995).

Karin, M., Mitogen-activated protein kinase cascades as regulators of stress responses. *Ann N Y Acad Sci,* 851, 139-146 (1998).

Kashfi, K., Yang, E. K., Chowdhury, J. R., Chowdhury, N. R. and Dannenberg, A. J., Regulation of uridine diphosphate glucuronosyltransferase expression by phenolic antioxidants. *Cancer Res,* 54, 5856-5859 (1994).

Kataoka, K., Noda, M. and Nishizawa, M., Maf nuclear oncoprotein recognizes sequences related to an AP-1 site and forms heterodimers with both Fos and Jun. *Mol Cell Biol,* 14, 700-712 (1994).

Katiyar, S. K., Agarwal, R. and Mukhtar, H., Protection against malignant conversion of chemically induced benign skin papillomas to squamous cell carcinomas in SENCAR mice by a polyphenolic fraction isolated from green tea. *Cancer Res,* 53, 5409-5412 (1993).

Kelloff, G. J., Boone, C. W., Steele, V. E., Crowell, J. A., Lubet, R. A., Greenwald, P., Hawk, E. T., Fay, J. R. and Sigman, C. C., Mechanistic considerations in the evaluation of chemopreventive data. *IARC Sci Publ,* , 203-219 (1996).

Kensler, T. W., Egner, P. A., Dolan, P. M., Groopman, J. D. and Roebuck, B. D., Mechanism of protection against aflatoxin tumorigenicity in rats fed 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) and related 1,2-dithiol-3-thiones and 1,2-dithiol-3-ones. *Cancer Res,*

47, 4271-4277 (1987).

Kensler, T. W., Groopman, J. D., Eaton, D. L., Curphey, T. J. and Roebuck, B. D., Potent inhibition of aflatoxin-induced hepatic tumorigenesis by the monofunctional enzyme inducer 1,2-dithiole-3-thione. *Carcinogenesis,* 13, 95-100 (1992).

Kim, N. D. and Kim, S. G., Chemopreventive effects of 2-(allylthio)pyrazine. *Arch Pharm Res,* 22, 99-107 (1999).

King, M. M. and McCay, P. B., Modulation of tumor incidence and possible mechanisms of inhibition of mammary carcinogenesis by dietary antioxidants. *Cancer Res,* 43, 2485s-2490s (1983).

Kirlin, W. G., Cai, J., DeLong, M. J., Patten, E. J. and Jones, D. P., Dietary compounds that induce cancer preventive phase 2 enzymes activate apoptosis at comparable doses in HT29 colon carcinoma cells. *J Nutr,* 129, 1827-1835 (1999).

Kitchen, I., Tremblay, J., Andre, J., Dring, L. G., Idle, J. R., Smith, R. L. and Williams, R. T., Interindividual and interspecies variation in the metabolism of the hallucinogen 4-methoxyamphetamine. *Xenobiotica,* 9, 397-404 (1979).

Kluck, R. M., Bossy-Wetzel, E., Green, D. R. and Newmeyer, D. D., The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis [see comments]. *Science,* 275, 1132-1136 (1997).

Kyriakis, J. M. and Avruch, J., Protein kinase cascades activated by stress and inflammatory cytokines. *Bioessays,* 18, 567-577 (1996).

Kyriakis, J. M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E. A., Ahmad, M. F., Avruch, J. and Woodgett, J. R., The stress-activated protein kinase subfamily of c-Jun kinases. *Nature,* 369, 156-160 (1994).

Lam, L. K., Fladmoe, A. V., Hochalter, J. B. and Wattenberg, L. W., Short time interval effects of butylated hydroxyanisole on the metabolism of benzo(a)pyrene. *Cancer Res,* 40, 2824-2828 (1980).

Langman, M. and Boyle, P., Chemoprevention of colorectal cancer. *Gut,* 43, 578-585 (1998).

Lee, J. C., Laydon, J. T., McDonnell, P. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Heys, J. R., Landvatter, S. W. and et al., A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature,* 372, 739-746 (1994).

Lei, W., Yu, R., Mandlekar, S. and Kong, A. N., Induction of apoptosis and activation of interleukin 1beta-converting enzyme/Ced-3 protease (caspase-3) and c-Jun NH2-terminal kinase 1 by benzo(a)pyrene. *Cancer Res,* 58, 2102-2106 (1998).

Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S. M., Ahmad, M., Alnemri, E. S. and Wang, X., Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell,* 91, 479-489 (1997).

Li, Y. and Jaiswal, A. K., Regulation of human NAD (P)H:quinone oxidoreductase gene. Role of AP1 binding site contained within human antioxidant response element [published erratum appears in J Biol Chem 1993 Oct 5;268(28):21454]. *J Biol Chem,* 267, 15097-15104 (1992).

Lin, J. K., Liang, Y. C. and Lin-Shiau, S. Y., Cancer chemoprevention by tea polyphenols through mitotic signal transduction blockade. *Biochem Pharmacol,* 58, 911-915 (1999).

Liu, X., Zou, H., Slaughter, C. and Wang, X., DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell,* 89, 175-184 (1997).

Lotan, R., Retinoids in cancer chemoprevention. *Faseb J,* 10, 1031-1039 (1996).

Lupulescu, A. P., Hormones, vitamins, and growth factors in cancer treatment and prevention. A critical appraisal. *Cancer,* 78, 2264-2280 (1996).

Mackenzie, P. I., Owens, I. S., Burchell, B., Bock, K. W., Bairoch, A., Belanger, A., Fournel-Gigleux, S., Green, M., Hum, D. W., Iyanagi, T., Lancet, D., Louisot, P., Magdalou, J., Chowdhury, J. R., Ritter, J. K., Schachter, H., Tephly, T. R., Tipton, K. F. and Nebert, D. W., The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics,* 7, 255-269 (1997).

Marshall, C. J., MAP kinase kinase kinase, MAP kinase kinase and MAP kinase. *Curr Opin Genet Dev,* 4, 82-89 (1994).

Martin, S. J. and Green, D. R., Protease activation during apoptosis: death by a thousand cuts? *Cell,* 82, 349-352 (1995).

McGowan, A. J., Ruiz-Ruiz, M. C., Gorman, A. M., Lopez-Rivas, A. and Cotter, T. G., Reactive oxygen intermediate(s) (ROI): common mediator(s) of poly (ADP-ribose)polymerase (PARP) cleavage and apoptosis. *FEBS Lett,* 392, 299-303 (1996).

Mizutani, T., Nomura, H., Nakanishi, K. and Fujita, S., Hepatotoxicity of butylated hydroxytoluene and its analogs in mice depleted of hepatic glutathione. *Toxicol Appl Pharmacol,* 87, 166-176 (1987).

Moi, P., Chan, K., Asunis, I., Cao, A. and Kan, Y. W., Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proc Natl Acad Sci U S A,* 91, 9926-9930 (1994).

Moinova, H. R. and Mulcahy, R. T., Up-regulation of the human gamma-glutamylcysteine synthetase regulatory subunit gene involves binding of Nrf-2 to an electrophile responsive element. *Biochem Biophys Res Commun,* 261, 661-668 (1999).

Mulder, G., Coughtrie, MWH, and Burchell, B., Glucuronidation. In GJ, M., (Eds). *Conjugation Reactions in*

Drug Metabolism. Taylor & Francis, London, pp. 51-106, 1990.

Munzel, P. A., Schmohl, S., Heel, H., Kalberer, K., Bock-Hennig, B. S. and Bock, K. W., Induction of human UDP glucuronosyltransferases (UGT1A6, UGT1A9, and UGT2B7) by t-butylhydroquinone and 2,3,7,8-tetrachlorodibenzo-p-dioxin in Caco-2 cells. *Drug Metab Dispos,* 27, 569-573 (1999).

Nagata, S., Apoptosis by death factor. *Cell,* 88, 355-365 (1997).

Nelson, D. R., Kamataki, T., Waxman, D. J., Guengerich, F. P., Estabrook, R. W., Feyereisen, R., Gonzalez, F. J., Coon, M. J., Gunsalus, I. C., Gotoh, O. and et al., The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. *DNA Cell Biol,* 12, 1-51 (1993).

Okuda, A., Imagawa, M., Maeda, Y., Sakai, M. and Muramatsu, M., Structural and functional analysis of an enhancer GPEI having a phorbol 12-O-tetradecanoate 13-acetate responsive element-like sequence found in the rat glutathione transferase P gene. *J Biol Chem,* 264, 16919-16926 (1989).

Pascale, R. M., Marras, V., Simile, M. M., Daino, L., Pinna, G., Bennati, S., Carta, M., Seddaiu, M. A., Massarelli, G. and Feo, F., Chemoprevention of rat liver carcinogenesis by S-adenosyl-L-methionine: a long-term study. *Cancer Res,* 52, 4979-4986 (1992).

Patten, E. J. and DeLong, M. J., Temporal effects of the detoxification enzyme inducer, benzyl isothiocyanate: activation of c-Jun N-terminal kinase prior to the transcription factors AP-1 and NFkappaB. *Biochem Biophys Res Commun,* 257, 149-155 (1999).

Pezzuto, J., *Cancer chemopreventive agents: from plant materials to clinical intervention trials.* American Chemical Society Books, Washington, DC, 1993.

Pezzuto, J., *Natural product cancer chemopreventive agents*. Plenum Press, New York, 1995.

Pezzuto, J. M., Plant-derived anticancer agents. *Biochem Pharmacol,* 53, 121-133 (1997).

Pinkus, R., Weiner, L. M. and Daniel, V., Role of oxidants and antioxidants in the induction of AP-1, NF-kappaB, and glutathione S-transferase gene expression. *J Biol Chem,* 271, 13422-13429 (1996).

Prestera, T., Holtzclaw, W. D., Zhang, Y. and Talalay, P., Chemical and molecular regulation of enzymes that detoxify carcinogens. *Proc Natl Acad Sci U S A,* 90, 2965-2969 (1993).

Prochaska, H. J. and Talalay, P., Regulatory mechanisms of monofunctional and bifunctional anticarcinogenic enzyme inducers in murine liver. *Cancer Res,* 48, 4776-4782 (1988).

Raingeaud, J., Gupta, S., Rogers, J. S., Dickens, M., Han, J., Ulevitch, R. J. and Davis, R. J., Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *J Biol Chem,* 270, 7420-7426 (1995).

Rouse, J., Cohen, P., Trigon, S., Morange, M., Alonso-Llamazares, A., Zamanillo, D., Hunt, T. and Nebreda, A. R., A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. *Cell,* 78, 1027-1037 (1994).

Rushmore, T., Pickett, CB, and Lu AYH., *Regulation fo expression of rat liver glutathione S-transferases: Xenobiotic and antioxidant induction of the Ya subunit gene.* Springer-Verlag, Berlin Heidelberg, 1994.

Rushmore, T. H., Morton, M. R. and Pickett, C. B., The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. *J Biol Chem,* 266, 11632-11639 (1991).

Rushmore, T. H. and Pickett, C. B., Transcriptional regulation of the rat glutathione S-transferase Ya subunit gene. Characterization of a xenobiotic-responsive element controlling inducible expression by phenolic antioxidants. *J Biol Chem,* 265, 14648-14653 (1990).

Samaha, H. S., Kelloff, G. J., Steele, V., Rao, C. V. and Reddy, B. S., Modulation of apoptosis by sulindac, curcumin, phenylethyl-3- methylcaffeate, and 6-phenylhexyl isothiocyanate: apoptotic index as a biomarker in colon cancer chemoprevention and promotion. *Cancer Res,* 57, 1301-1305 (1997).

Shao, Z. M., Wu, J., Shen, Z. Z. and Barsky, S. H., Genistein exerts multiple suppressive effects on human breast carcinoma cells. *Cancer Res,* 58, 4851-4857 (1998).

Skolnik, E. Y., Lee, C. H., Batzer, A., Vicentini, L. M., Zhou, M., Daly, R., Myers, M. J., Jr., Backer, J. M., Ullrich, A., White, M. F. and et al., The SH2/SH3 domain-containing protein GRB2 interacts with tyrosine-phosphorylated IRS1 and Shc: implications for insulin control of ras signalling. *Embo J,* 12, 1929-1936 (1993).

Slater, A., Stefan, C, Nobel, I, Van Den Dobbelsteen, DJ, and Orrenius, S., Intracellular redox changes during apoptosis. *Cell Death and Differentiation,* 3, 57-62 (1996).

Smith, C. A., Farrah, T. and Goodwin, R. G., The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. *Cell,* 76, 959-962 (1994).

Sparnins, V. L., Chuan, J. and Wattenberg, L. W., Enhancement of glutathione S-transferase activity of the esophagus by phenols, lactones, and benzyl isothiocyanate. *Cancer Res,* 42, 1205-1207 (1982a).

Sparnins, V. L., Venegas, P. L. and Wattenberg, L. W., Glutathione S-transferase activity: enhancement by compounds inhibiting chemical carcinogenesis and by

dietary constituents. *J Natl Cancer Inst,* 68, 493-496 (1982b).

Steller, H., Mechanisms and genes of cellular suicide. *Science,* 267, 1445-1449 (1995).

Stokoe, D., Macdonald, S. G., Cadwallader, K., Symons, M. and Hancock, J. F., Activation of Raf as a result of recruitment to the plasma membrane [see comments] [published erratum appears in Science 1994 Dec 16;266(5192):1792-3]. *Science,* 264, 1463-1467 (1994).

Stoner, G. D., Morrissey, D. T., Heur, Y. H., Daniel, E. M., Galati, A. J. and Wagner, S. A., Inhibitory effects of phenethyl isothiocyanate on N- nitrosobenzylmethylamine carcinogenesis in the rat esophagus. *Cancer Res,* 51, 2063-2068 (1991).

Talalay, P., De Long, M.J., and Prochaska, H.J., *Molecular Mechanisms in protection against carcinogenesis.* Plenum, New York, 1987.

Talalay, P., Mechanisms of induction of enzymes that protect against chemical carcinogenesis. *Adv Enzyme Regul,* 28, 237-250 (1989).

Tartaglia, L. A., Rothe, M., Hu, Y. F. and Goeddel, D. V., Tumor necrosis factor's cytotoxic activity is signaled by the p55 TNF receptor. *Cell,* 73, 213-216 (1993).

Tewari, M., Quan, L. T., K, O. R., Desnoyers, S., Zeng, Z., Beidler, D. R., Poirier, G. G., Salvesen, G. S. and Dixit, V. M., Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell,* 81, 801-809 (1995).

Thompson, C. B., Apoptosis in the pathogenesis and treatment of disease. *Science,* 267, 1456-1462 (1995).

Thornberry, N. A. and Lazebnik, Y., Caspases: enemies within. *Science,* 281, 1312-1316 (1998).

Ulland, B. M., Weisburger, J. H., Yamamoto, R. S. and Weisburger, E. K., Antioxidants and carcinogenesis: butylated hydroxytoluene, but not diphenyl-p-phenylenediamine, inhibits cancer induction by N-2-fluorenylacetamide and by N-hydroxy-N-2-fluorenylacetamide in rats. *Food Cosmet Toxicol,* 11, 199-207 (1973).

Venugopal, R. and Jaiswal, A. K., Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. *Proc Natl Acad Sci U S A,* 93, 14960-14965 (1996).

Verheij, M., Bose, R., Lin, X. H., Yao, B., Jarvis, W. D., Grant, S., Birrer, M. J., Szabo, E., Zon, L. I., Kyriakis, J. M., Haimovitz-Friedman, A., Fuks, Z. and Kolesnick, R. N., Requirement for ceramide-initiated SAPK/JNK signalling in stress-induced apoptosis. *Nature,* 380, 75-79 (1996).

Wang, X. S., Diener, K., Manthey, C. L., Wang, S., Rosenzweig, B., Bray, J., Delaney, J., Cole, C. N., Chan-Hui, P. Y., Mantlo, N., Lichenstein, H. S., Zukowski, M. and Yao, Z., Molecular cloning and characterization of a novel p38 mitogen-activated protein kinase. *J Biol Chem,* 272, 23668-23674 (1997).

Wang, Z. Y., Cheng, S. J., Zhou, Z. C., Athar, M., Khan, W. A., Bickers, D. R. and Mukhtar, H., Antimutagenic activity of green tea polyphenols. *Mutat Res,* 223, 273-285 (1989).

Wang, Z. Y., Huang, M. T., Lou, Y. R., Xie, J. G., Reuhl, K. R., Newmark, H. L., Ho, C. T., Yang, C. S. and Conney, A. H., Inhibitory effects of black tea, green tea, decaffeinated black tea, and decaffeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7,12-dimethylbenz[a]anthracene-initiated SKH-1 mice. *Cancer Res,* 54, 3428-3435 (1994).

Wasserman, W. W. and Fahl, W. E., Functional antioxidant responsive elements. *Proc Natl Acad Sci U S A,* 94, 5361-5366 (1997).

Wattenberg, L. W., Inhibition of chemical carcinogen-induced pulmonary neoplasia by butylated hydroxyanisole. *J Natl Cancer Inst,* 50, 1541-1544 (1973).

Wattenberg, L. W., Inhibition of carcinogenic effects of polycyclic hydrocarbons by benzyl isothiocyanate and related compounds. *J Natl Cancer Inst,* 58, 395-398 (1977).

Wattenberg, L. W., Inhibition of neoplasia by minor dietary constituents. *Cancer Res,* 43, 2448s-2453s (1983).

Wattenberg, L. W., Chemoprevention of cancer. *Cancer Res,* 45, 1-8 (1985).

Wattenberg, L. W., Protective effects of 2(3)-tert-butyl-4-hydroxyanisole on chemical carcinogenesis. *Food Chem Toxicol,* 24, 1099-1102 (1986).

Wattenberg, L. W., Prevention--therapy--basic science and the resolution of the cancer problem. *Cancer Res,* 53, 5890-5896 (1993).

Wattenberg, L. W. and Bueding, E., Inhibitory effects of 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (Oltipraz) on carcinogenesis induced by benzo[a]pyrene, diethylnitrosamine and uracil mustard. *Carcinogenesis,* 7, 1379-1381 (1986).

Wattenberg, L. W., Coccia, J. B. and Lam, L. K., Inhibitory effects of phenolic compounds on benzo(a)pyrene-induced neoplasia. *Cancer Res,* 40, 2820-2823 (1980).

Wattenberg, L. W., Lam, L. K., Fladmoe, A. V. and Borchert, P., Inhibits of of colon carcinogenesis. *Cancer,* 40, 2432-2435 (1977).

Weinshilboum, R. M., Otterness, D. M., Aksoy, I. A., Wood, T. C., Her, C. and Raftogianis, R. B., Sulfation and sulfotransferases 1: Sulfotransferase molecular biology: cDNAs and genes. *Faseb J,* 11, 3-14 (1997).

Weinstein, I. B., Begemann, M., Zhou, P., Han, E. K., Sgambato, A., Doki, Y., Arber, N., Ciaparrone, M. and Yamamoto, H., Disorders in cell circuitry associated with multistage carcinogenesis: exploitable targets for cancer prevention and therapy. *Clin Cancer Res,* 3, 2696-2702 (1997).

Weisburger, E. K. and Evarts, R. P., Inhibitory effect of butylated hydroxytoluene (BHT) on intestinal carcinogenesis in rats by azoxymethane. *Food Cosmet Toxicol,* 15, 139-141 (1977).

Williams, R. T., Species variations in the pathways of drug metabolism. *Environ Health Perspect,* 22, 133-138 (1978).

Wormhoudt, L. W., Commandeur, J. N. and Vermeulen, N. P., Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione-S-transferase, and epoxide hydrolase enzymes: relevance to xenobiotic metabolism and toxicity. *Crit Rev Toxicol,* 29, 59-124 (1999).

Xu, Y., Ho, C. T., Amin, S. G., Han, C. and Chung, F. L., Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants. *Cancer Res,* 52, 3875-3879 (1992).

Yan, Y. S., [Effect of Chinese green tea extracts on the human gastric carcinoma cell in vitro]. *Chung Hua Yu Fang I Hsueh Tsa Chih,* 24, 80-82 (1990).

Yang, C. S., Brady, J. F. and Hong, J. Y., Dietary effects on cytochromes P450, xenobiotic metabolism, and toxicity. *Faseb J,* 6, 737-744 (1992).

Yang, C. S., Smith, T. J. and Hong, J. Y., Cytochrome P-450 enzymes as targets for chemoprevention against chemical carcinogenesis and toxicity: opportunities and limitations. *Cancer Res,* 54, 1982s-1986s (1994).

Yang, C. S. and Wang, Z. Y., Tea and cancer. *J Natl Cancer Inst,* 85, 1038-1049 (1993).

Yang, G. Y., Liao, J., Kim, K., Yurkow, E. J. and Yang, C. S., Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. *Carcinogenesis,* 19, 611-616 (1998).

Yang, J., Liu, X., Bhalla, K., Kim, C. N., Ibrado, A. M., Cai, J., Peng, T. I., Jones, D. P. and Wang, X., Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked [see comments]. *Science,* 275, 1129-1132 (1997).

Ye, H., Kelly, T. F., Samadani, U., Lim, L., Rubio, S., Overdier, D. G., Roebuck, K. A. and Costa, R. H., Hepatocyte nuclear factor 3/fork head homolog 11 is expressed in proliferating epithelial and mesenchymal cells of embryonic and adult tissues. *Mol Cell Biol,* 17, 1626-1641 (1997).

Yu, R., Mandlekar, S., Harvey, K.J., Ucker, D.S., and Kong, A.-N.T., Chemopreventive isothiocyanates induce apoptosis and caspase-3-like protease activity. *Cancer Res.,* 58, 402-408 (1998a).

Yu, R., Mandlekar, S., and Kong, A.-N.T. , Distinct roles of intracellular calcium in the activation of c-jun N-terminal kinases and caspases during apoptosis induced by phenolic antioxidant BHA. In *37th Annual Meeting of Society of Toxicology, Seattle, WA, Toxicological Sciences (Formerly Fund. Appl. Tox.).* pp. 356. Seattle, WA (1998b).

Yu, R., Mandlekar, S., Lei, W., Fahl, W.E., Tan, T.-H., and Kong, A.-N.T., p38 mitogen-activated protein kinase negatively regulates the induction of phase II drug metabolizing enzymes that detoxify carcinogens. *J. Biol. Chem.,* 275 (February 2000 (in the press)).

Yu, R., Jiao, J. J., Duh, J. L., Gudehithlu, K., Tan, T. H. and Kong, A. N. T., Activation of mitogen-activated protein kinases by green tea polyphenols: potential signaling pathways in the regulation of antioxidant-responsive element-mediated phase II enzyme gene expression. *Carcinogenesis,* 18, 451-456 (1997a).

Yu, R., Jiao, J. J., Duh, J. L., Tan, T. H. and Kong, A. N. T., Phenethyl isothiocyanate, a natural chemopreventive agent, activates c-Jun N-terminal kinase 1. *Cancer Res,* 56, 2954-2959 (1996a).

Yu, R., Lei, W., Mandlekar, S., Weber, M. J., Der, C. J., Wu, J. and Kong, A. T., Role of a Mitogen-activated Protein Kinase Pathway in the Induction of Phase II Detoxifying Enzymes by Chemicals. *J Biol Chem,* 274, 27545-27552 (1999).

Yu, R., Shtil, A. A., Tan, T. H., Roninson, I. B. and Kong, A. N. T., Adriamycin activates c-jun N-terminal kinase in human leukemia cells: a relevance to apoptosis. *Cancer Lett,* 107, 73-81 (1996b).

Yu, R., Tan, T. H. and Kong, A. T., Butylated hydroxyanisole and its metabolite tert-butylhydroquinone differentially regulate mitogen-activated protein kinases. The role of oxidative stress in the activation of mitogen-activated protein kinases by phenolic antioxidants. *J Biol Chem,* 272, 28962-28970 (1997b).

Zhang, Y., Kolm, R. H., Mannervik, B. and Talalay, P., Reversible conjugation of isothiocyanates with glutathione catalyzed by human glutathione transferases. *Biochem Biophys Res Commun,* 206, 748-755 (1995).

Zhang, Y. and Talalay, P., Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. *Cancer Res.,* 54, 1976s-1981s (1994).

Zhang, Y., Talalay, P., Cho, C. G. and Posner, G. H., A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc Natl Acad Sci U S A,* 89, 2399-2403 (1992).