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Antioxidants and oxidants regulated signal transduction pathways☆

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Abstract

Many drugs and xenobiotics induce signal transduction events leading to gene expression of either pharmacologically beneficial effects, or unwanted side effects such as cytotoxicity which can compromise drug therapy. Using dietary chemopreventive compounds (isothiocyanates and green tea polyphenols), which are effective against various chemically-induced carcinogenesis models in animals studies, we studied the signal transduction events and gene expression profiles. These compounds have typically generated cellular ''oxidative stress'' and modulated gene expression including phase II detoxifying enzymes GST and QR as well as cellular defensive enzymes, heme oxygenase 1 (HO-1) and *GST via* the antioxidant/electrophile response element (ARE/EpRE). Members of the bZIP transcription factor, Nrf2 which heterodimerizes with Maf G/K, were found to bind to ARE, and transcriptionally activate ARE. Additionally the mitogen-activated protein kinases (MAPK; ERK, JNK and p38) were differentially activated by these compounds, and involved in the transcriptional activation of ARE-mediated reporter gene. Transfection studies with various cDNA encoding for wild-type of MAPK and Nrf2 showed synergistic response during co-transfection and to these agents. However, by increasing the concentrations of these xenobiotics, caspase activities and apoptosis were observed which were preceded by mitochondria damage and cytochrome *c* mitochondria release. Further, increased concentrations led to rapid cell necrosis. DNA microarray analyses were performed to ascertain the gene expression profiles elicited by these compounds at low concentrations as well as at higher concentrations. Thus, we have proposed a model, that at low concentrations, these compounds activate MAPK pathway leading to activation of Nrf2 and ARE with subsequent induction of phase II and other defensive genes which protect cells against toxic insults thereby enhancing cell survival, a beneficial homeostatic response. At higher concentrations, these agents activate the caspase pathways, leading to apoptosis, a potential cytotoxic effect if it occurred in normal cells. The studies of these signaling pathways may yield important insights into the pharmacodynamic and toxicodynamic effects of drugs and xenobiotics during pharmaceutical drug discovery and development.

Keywords: Phenolic antioxidants; Gene expression; Mitogen-activated protein kinase; Caspases; Apoptosis

1. Introduction

Components of natural and synthetic compounds are known to be potent in cancer chemopreventive properties, in chemically-induced carcinogenesis models. These cancer chemopreventive agents are very promising in that they offer a non-toxic route through intervention on processes of carcinogenesis. However, understanding the processes that lead to carcinogenesis requires a clear identification of molecular targets of cancer causing agents. This is fundamental in designing effective clinical cancer therapy programs. The lack of understanding of signal transduction events that are mobilized by molecular targets upon activation by the cancer causing agents, is a major impediment in designing cancer therapeutic and chemopreventive

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Abbreviations: BHA, 2(3)-*tert*-butyl-4-hydroxyanisole; tBHQ, *tert*-butyl-hydroquinone; GTP, green tea polyphenols; EGCG, (−)-epigallocatechin-3-gallate; ECG, (−)-epicatechin-3-gallate; PEITC, phenethyl-isothiocyanate; SUL, sulforaphane; MAPK, mitogen-activated protein kinase; ERK2, extracellular signal-regulated protein kinase 2; ERK5, extracellular signal-regulated protein kinase 5; BMK1, big mitogen-activated protein kinase 1; JNK, c-*jun* N-terminal kinase; TAK1, transforming growth factor-β-activated kinase 1; ASK1, apoptosis signal-regulating kinase; MKK, MAPK kinase; Nrf2, NF-E2-related factor 2; ARE/EpRE, antioxidant/electrophile response element; Cyto *c*, cytochrome *c*; GST, glutathione *S*-transferase; QR, quinone reductase; HO-1, heme oxygenase 1.

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agents. In this review, we have characterized signal transduction events that are turned-on by two classes of potential cancer chemopreventive compounds, namely phenolic compounds/antioxidants and isothiocyanates. This review examines the potential utility of mitogen-activated protein kinases (MAPKs), the ICE/Ced-3 family proteases (caspases), and their modulation by these compounds in the decision process for cell life or death. The modulation of cellular survival signaling pathways vs. cell death pathways has significant biological consequences that are important in developing predictions on various pharmacological and toxicological responses.

2. Modulation of MAPK

MAPKs are largely characterized as proline-directed serine/threonine (Pro*X*Ser/ThrPro); [1,2] kinases [3], that are important signaling components in the conversion of extracellular signals into intracellular responses, through serial phosphorylation cascades [4]. The ultimate effects of MAPK activation and phosphorylations depend on their ability to induce the appropriate gene expression events as a homeostatic response within the cell. Some of these genes are responsible for cellular defense or programmed cell death through the expression of either anti- or pro-apoptotic proteins. Three MAP kinase pathways, ERK, JNK, p38 have been identified and well studied [5,6]. Each MAPK pathway 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. Among the MAP kinases, ERK is mainly activated by mitogens and growth factors, while p38 and JNK are activated by many environmental stress stimuli (e.g. UV, ionizing radiation), resulting in apoptosis [7].

Several chemotherapeutic drugs have been shown to activate JNK and p38 MAPK, and their activation implicated in apoptosis [8,9]. Once activated, ERK, JNK, and p38 phosphorylate many transcription factors, such as Nrf2, c-*myc*, p62TCF/Elk-1, c-*jun*, ATF2, CHOP/GADD153, MEF2C, and SAP-1, ultimately leading to the changes in gene expression profiles with biological consequences [10,11]. Because of their ability to respond to a variety of stimuli, MAP kinase signaling cascades are capable of activating similar, but greatly integrated mechanisms that characterize cross-talk between signaling pathways, with the common end point of transducing specific cellular responses (gene expression) to various extracellular stimuli, including oxidative stress and pharmacological agents. We have recently reported, in studies examining ERK5/Big MAP kinase 1 (BMK1) signaling in human squamous cervical carcinoma cells, that phenethyl-isothiocyanates (PEITC)-induced activation of Nrf2-dependent ARE genes for cellular defense manifests significant cross-talk with p38 pathway, a cell death pathway (unpublished data). This emphasized the complexity of MAPK signaling, the result of which depends on what signal out titrates the other.

3. MAPK pathways mediate ARE-dependent induction of detoxifying enzymes

Phenolic antioxidant butylated hydroxyanisol (BHA) and its demethylated metabolite *tert*-butylhydroquinone (tBHQ) as well as the electrophilic dietary compounds the isothiocyanates such as PEITC and sulforaphane (SUL), are highly effective cancer chemopreventive agents. These agents have been shown to act against tumor formation by a variety of carcinogens in animal models of human cancers [12–14]. These compounds are also known to be potent inducers of phase II detoxifying enzymes, such as glutathione *S*-transferease (GST) and quinone reductase (QR) [15–17]. In studies, animals treated with BHA, tBHQ, PEITC, and other potential chemopreventive agents that are phase II xenobiotic detoxifying enzyme-inducers, displayed a remarkably enhanced ability to convert chemical carcinogens (e.g. aflatoxin B1 and benzo[a]pyrene) to their non-mutagenic metabolites, thereby facilitating their detoxification and excretion through their conjugated derivatives. Consequently, treatment with these chemopreventive agents dramatically reduces toxicity, mutagenicity, and carcinogenicity by various chemical carcinogens [18,19].

Our laboratory has shown that BHA potently activates JNK1 and ERK2 activities in many mammalian and murine cells [20] in a time- and dose-dependent manner. Similarly, tBHQ, the demethylated active metabolite of BHA and a potent inducer of phase II genes [21–23], stimulated the activity of ERK2, but weakly activated JNK [20,24]. Exposure of cells to green tea polyphenols (GTP) potently activated JNK1 and ERK2 activities with different kinetic- and dose-responses [25].

Activation of MAPK by GTP and isothiocyanates, among others, also induced mRNA expression of immediate early genes such as c-*jun*, and c-*fos*, and transcriptionally activated the antioxidant/electrophile response element (ARE/EpRE), chloramphenicol acetyltransferase (CAT) reporter gene, present in many stress-response genes [26], including phase II drug metabolizing enzymes like GST, QR, [27–29], and heme oxygenase 1 (HO-1), which encode for defense against cellular oxidative stress [30]. In our studies, we demonstrated potency of (−)-epigallocatechine-3-gallate (EGCG) and (−)-epicatechin-3-gallate (ECG) in inducing ARE-mediated luciferase activity, and ERK, JNK and p38 MAPK [31]. Furthermore, most recently, we have shown that ERK5/BMK1 activates ARE luciferase activity, and that this activation is regulated upstream by Raf-1, MEKK1, TAK1.

In examining the downstream physiological consequences of MAPK activation by chemopreventive compounds, we recently, found that ERK2, ERK5 and JNK MAP kinase cascades show a positive transcriptional

activation of ARE-mediated reporter gene induced by tBHQ, PEITC, and SUL [32], whereas activation of the p38 MAPK negatively regulated ARE-activity [33]. This suggested a coordinate modulation of MAPK cascades by reactive intermediates including reactive oxidative stress (ROS), which may be critical in the regulation of phase II genes through the ARE/EpRE DNA enhancer element during cellular homeostasis.

4. Transcription factor Nrf2 modulates expression of phase II detoxifying genes

Recently, several groups have shown that during oxidative stress, the basic leucine zipper (bZIP) transcription factors, including Nrf1 [34], Nrf2 [34,35] heterodimerize with small Maf [35] and bind ARE sequences thereby transcriptionally activating ARE. We have also shown that oxidative stress-induced expression of MAP kinases such as Raf-1, MEKK1, ASK1, TAK1 enormously enhance the Nrf2 transcriptional activity (Fig. 1). Thus, in elucidating the link between members of the MAP kinase and Nrf2 transcription factor, we have examined the role of a panel of MAP kinases in phosphorylating Nrf2. In this study, we recently showed that Nrf2 was phosphorylated by ERK5, thereby implying that MAP kinases transduce extracellular signals into gene action through the intermediary of transcription factors like Nrf2, and that phosphorylation might be a major mechanism for achieving appropriate gene expression.

Further to understand the targeting of cellular stress-response, we examined the distribution and localization of Nrf2 with and without oxidative stress. Though still obscure, two models have been proposed to explain how Nrf2 senses oxidative stress. The initially suggested model showed that the relative distribution of Nrf2 between the cytosol and the nucleus is in many respects similar to that of Iκ-B/NF-κB system. This model suggests that a cytosolic protein, Keap1, suppresses Nrf2 transcriptional activity by coupling and retaining Nrf2 in the cytosol [36]. Upon activation by oxidative stress, the Keap1–Nrf2 complex uncouples through the phosphorylation of Nrf2, and Nrf2 translocates into the nucleus, binds to a specific DNA sequence thereby leading to the expression of genes encoding ARE-dependent detoxifiers. However, through a series of studies examining both nuclear and cytosolic Nrf2, we proposed an alternative model in which most of Nrf2 is nuclear, and not cytosolic before and during oxidative stress (unpublished data). This, to us, strongly suggested that, depending on the external stimulus, different tissues differentially modulate Nrf2 localization and activity as an oxidative stress sensor. The role of Nrf2 as a cellular stress sensor cannot be over emphasized since Nrf2−/− mice knock-out eliminated induction of QR and GST [35,37] Nrf2. To understand how Nrf2 regulates ARE-dependent genes, we demonstrate a dose-dependent increase in ARE-activity with increasing Nrf2 [32]. The dominant negative deletion mutant of Nrf2 abolished transcriptional activation of ARE-LUC reporter gene [32].

5. Apoptosis and cancer chemoprevention

Programmed cell death is known to be under a very tight genetic control with evolutionarily conserved molecular mechanisms between species. This process plays important

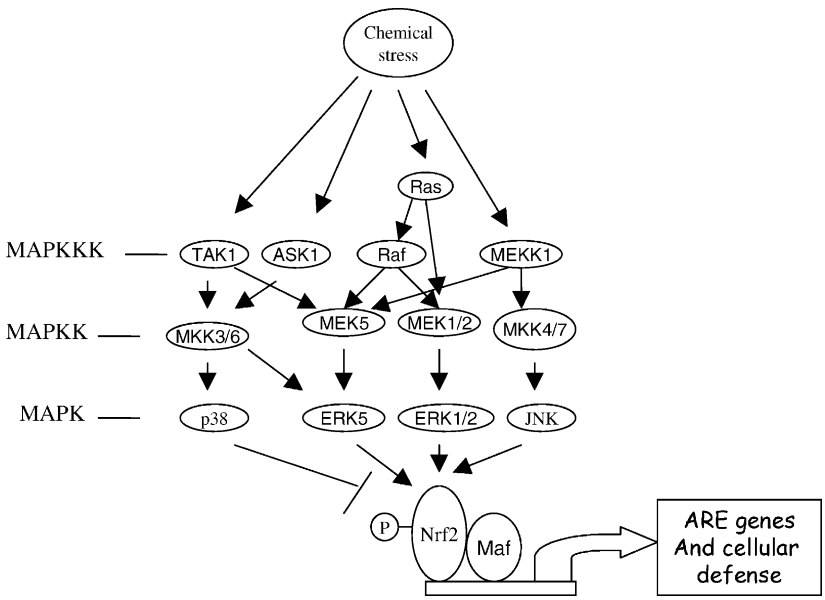

Fig. 1. A model of drugs/chemicals/xenobiotics-induced chemical stress response leading to the activation of MAPK signaling pathway and subsequent activation of Nrf2/Maf-dependent anti-oxidant responsive genes including the phase II detoxifying enzymes as well as other stress enzymes, which result in the enhancement of detoxification of the xenobiotics and a potential homeostatic cell survival response.

roles in many biological processes including carcinogenesis, tumorigenesis and cancer. Apoptosis may play a central homeostatic role by which genetically-damaged cells are deleted from the body, positive and negative selection in immune systems, and elimination of virally infected cells. Apoptosis of pre-initiated and/or neoplastic cells represents a protective mechanism against neoplastic transformation and development of tumors by the elimination of genetically damaged cells or cells that may have been inappropriately induced to divide by mitogenic and proliferative stimuli. Apoptosis is typically induced by the activation of membrane receptors [38], inhibition of protein kinases by staurosporine, cell cycle arrest and p53 activation by DNA damaging agents, mitochondria membrane pore transition permeability (MPT) by oxidants. Death receptors transmit apoptotic signals initiated on the plasma membrane by specific ''death ligands'' thereby playing a central role in programmed cell death [38]. After the binding of an appropriate ligand to the receptor, a receptor activated complex recruits intracellular domains and adaptor proteins, thereby transmitting the death signal. This is accomplished by activating downstream intracellular apoptotic machinery such as the ICE/Ced-3 family proteases (caspases). 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 [39–42].

6. Electrophiles-induced mitochondrial events

The mitochondria is a very central apparatus in cell death signaling through its ability to differentially regulate the trafficking of pro- and anti-apoptotic proteins, such as Bcl-2 [43,44], within its inter-membrane space, depending on the type of stimulus. The release of cytochrome *c* (cyto *c*) is known to be *via* two main mechanisms; MPT-dependent and MPT-independent mechanisms. In the MPT-dependent mechanism, the mitochondrial pores open followed by the movement of cyto *c* from the inter-membrane space, then complexes with apoptotic protease activating factor 1 (Apaf-1), ATP and procaspase-9 to form the apoptosome [45]. Several agents have the ability to induce MPT, and these include oxidants. They lead to formation of the apoptosome, activation of caspase-9, which in turn activates downstream caspase-3, a hallmark of apoptosis. Caspase-6 activation then drives the caspase cascade hence keeping the cell death mechanism as is necessary. Despite being unclear, the release of cyto *c* is postulated to either occur as a continuous process, or to be triggered by induction of MPT, or to occur with the swelling of the mitochondria, or by a specific chaperon or to be under the influence of some specific pore mechanism, or by a combination of these factors, thereby constituting the biochemical mechanisms for oxidant-induced cell death [46]. There is also mounting evidence suggesting that certain oxidants invoke the swelling of the mitochondria; thereby, causing it to burst and release proteins that activate the cell death mechanism. The release of anti-apoptotic Bcl-2 protein family (Bcl-2 and BclxL), is known to regulate apoptosis through inhibition of cyto *c* and Apaf-1 release by mechanisms that though still unclear, may involve channel formation activity and displacement of Apaf-1 from a complex of proteins. It is also proposed that anti- and pro-apoptotic proteins may posses a potential to heterodimerize thereby titrating one another's functions.

Though many cancer chemotherapeutic drugs induce apoptosis [9,47], several chemopreventive agents like PEITC, EGCG, chrysin, also induce apoptosis [48–58]. Induction of apoptosis by these agents is reported to be partially responsible for their chemopreventive activities [52–56]. To this effect, we studied the role of mitochondrial cyto *c* release, caspase activation pathways induced by BHA, among others and observed that BHA-induced apoptosis triggered MPT, which was inhibited by cyclosporin A [59]. Isothiocyanates such as PEITC, PMITC [60] or benzyl-isothiocyante (BIT) [61], also activated caspase-3 activity leading to apoptosis. Interestingly, the kinetics of activation of caspase activities induced by BHA or PEITC were rather rapid and apoptosis occurred shortly after, while EGCG was delayed [31]. Similar early response kinetics were observed for quercetin, chrysin, tamoxifen- and its two active metabolites (4-OH and N-desmethyl)-induced apoptosis in human estrogen independent BT-20 and MB-MD231 breast cancer cells [62]. Delayed caspase activity and apoptosis was also observed *in vivo* in rat mammary tumors after i.p. administrations of tamoxifen [63].

7. Discussion

In summary, our studies with various chemopreventive agents including tamoxifen, flavonoids, and various structurally related isothiocyanates, as well as phenolic antioxidants, provide important insights into the signal transduction pathways induced by these compounds. Low concentrations of these compounds activated MAPK pathways leading to the induction of phase II detoxifying enzymes induction for cellular protection signaling. Elevated concentrations of these compounds activated MAPK pathways, and the caspase pathways leading to apoptotic signaling. This concentration-dependent cellular response phenomenon represents a pharmacological window, above which toxicity sets in [64,65].

Several studies have shown that apigenin, silymarin, baicailein, resveratrol, EGCG, theaflavin, inhibit signaling kinases including MAPK and block AP-1-mediated gene expression. The exact mechanisms as to how these compounds inhibit kinase activation are not clear. Although

direct inhibition of upstream kinases such as c-*src* non-receptor kinase may contribute to the inhibition as seen with resveratrol [66]. Future studies are needed to explain these inhibitory mechanisms. Possibly in proliferating cells, these chemopreventive compounds may block proliferation by inhibiting signal transduction kinases, whereas in quiescent cells they activate signaling kinases leading to gene expression of cellular defensive. We observed that there are at least two groups of apoptotic-inducing chemopreventive agents, early apoptosis activators like isothiocyanates, phenolic antioxidants (2–4 hr) and delayed activators like tamoxifen, EGCG (12–18 hr). Understanding the mechanisms of induction of caspases by this latter group of compounds address this kinetic difference.

In conclusion, studies of cellular signal transduction events elicited by chemopreventive compounds may yield important insights into the regulation of gene expression for induction of phase II detoxifying enzymes and other cellular defensive enzymes such as HO-1. In contrast, activation of cell death proteins such as caspases, may potentially be beneficial if this occurs in preneoplastic or tumor cells, but may result in cytotoxicity if it occurs in normal cells. The small range of doses above which these chemopreventive agents become toxic represent the therapeutic windows *in vivo*. Consequently, signaling pathways will advance our understanding of efficacy and safety of many chemopreventive compounds with future therapeutic potential.

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References

[1] Gonzalez FA, Raden DL, Davis RJ. Identification of substrate recognition determinants for human ERK1 and ERK2 protein kinases. J Biol Chem 1991;266:22159–63.
[2] Alvarez E, Northwood IC, Gonzalez FA, Latour DA, Seth A, Abate C, Curran T, Davis RJ. 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 1991;266:15277–85.
[3] Marshall CJ. MAP kinase kinase kinase, MAP kinase kinase and MAP kinase. Curr Opin Genet Dev 1994;4:82–9.
[4] Cobb MH, Goldsmith EJ. How MAP kinases are regulated. J Biol Chem 1995;270:14843–6.
[5] Cano E, Mahadevan LC. Parallel signal processing among mammalian MAPKs. Trends Biochem Sci 1995;20:117–22.
[6] Kyriakis JM, Avruch J. Protein kinase cascades activated by stress and inflammatory cytokines. Bioessays 1996;18:567–77.
[7] Chen YR, Wang CX, Templeton D, Davis RJ, Tan TH. The role of c-jun N-terminal kinase (JNK) in apoptosis induced by ultraviolet C . J Biol Chem 1996;271:31929–36.
[8] Osborn MT, Chambers TC. Role of the stress-activated/c-jun NH2-terminal protein kinase pathway in the cellular response to adriamycin and other chemotherapeutic drugs. J Biol Chem 1996;271:30950–5.
[9] Hannun YA. Functions of ceramide in coordinating cellular responses to stress. Science 1996;274:1855–9.
[10] Karin M. The regulation of AP-1 activity by mitogen-activated protein kinases. J Biol Chem 1995;270:16483–6.
[11] Karin M. Mitogen-activated protein kinase cascades as regulators of stress responses. Ann N Y Acad Sci 1998;851:139–46.
[12] Wattenberg LW. Inhibition of chemical carcinogen-induced pulmonary neoplasia by butylated hydroxyanisole. J Natl Cancer Inst 1973; 50:1541–4.
[13] Wattenberg LW, Coccia JB, Lam LK. Inhibitory effects of phenolic compounds on benzo(a)pyrene-induced neoplasia. Cancer Res 1980;40:2820–3.
[14] King MM, McCay PB. Modulation of tumor incidence and possible mechanisms of inhibition of mammary carcinogenesis by dietary antioxidants. Cancer Res 1983;43:2485–90.
[15] Lam LK, Fladmoe AV, Hochalter JB, Wattenberg LW. Short time interval effects of butylated hydroxyanisole on the metabolism of benzo(a)pyrene. Cancer Res 1980;40:2824–8.
[16] Sparnins VL, Chuan J, Wattenberg LW. Enhancement of glutathione S-transferase activity of the esophagus by phenols, lactones, and benzyl isothiocyanate. Cancer Res 1982;42:1205–7.
[17] Sparnins VL, Venegas PL, Wattenberg LW. Glutathione S-transferase activity: enhancement by compounds inhibiting chemical carcinogenesis and by dietary constituents. J Natl Cancer Inst 1982;68:493–6.
[18] Batzinger RP, Ou SY, Bueding E. Antimutagenic effects of 2(3)-tert-butyl-4-hydroxyanisole and of antimicrobial agents. Cancer Res 1978;38:4478–85.
[19] Kensler TW, Egner PA, Dolan PM, Groopman JD, Roebuck BD. 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 1987;47: 4271–7.
[20] Yu R, Tan TH, Kong A-NT. 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 1997;272:28962–70.
[21] DeLong MJ, Prochaska HJ, Talalay P. Tissue-specific induction patterns of cancer-protective enzymes in mice by tert-butyl-4-hydroxyanisole and related substituted phenols. Cancer Res 1985;45: 546–51.
[22] Friling RS, Bensimon A, Tichauer Y, Daniel V. Xenobiotic-inducible expression of murine glutathione S-transferase Ya subunit gene is controlled by an electrophile-responsive element. Proc Natl Acad Sci USA 1990;87:6258–62.
[23] Rushmore TH, Morton MR, Pickett CB. The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. J Biol Chem 1991;266:11632–9.
[24] Yu R, Lei W, Mandlekar S, Weber MJ, Der CJ, Wu J, Kong A-NT. Role of a mitogen-activated protein kinase pathway in the induction of phase II detoxifying enzymes by chemicals. J Biol Chem 1999;274: 27545–52.
[25] Yu R, Jiao JJ, Duh JL, Gudehithlu K, Tan TH, Kong A-NT. 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 1997;18:451–6.
[26] Wasserman WW, Fahl WE. Functional antioxidant responsive elements. Proc Natl Acad Sci USA 1997;94:5361–6.
[27] Li Y, Jaiswal AK. Regulation of human NAD(P)H: quinone oxidoreductase gene. Role of AP1 binding site contained within human antioxidant response element. J Biol Chem 1992;267:15097–104.

[28] Rushmore TH, Pickett CB. 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 1990;265:14648–53.
[29] Prestera T, Holtzclaw WD, Zhang Y, Talalay P. Chemical and molecular regulation of enzymes that detoxify carcinogens. Proc Natl Acad Sci USA 1993;90:2965–9.
[30] Alam J, Stewart D, Touchard C, Boinapally S, Choi AM, Cook JL. Nrf2, a cap'n'collar transcription factor, regulates induction of the heme oxygenase-1 gene. J Biol Chem 1999;274:26071–8.
[31] Chen C, Yu R, Owuor ED, Kong A-NT. Activation of antioxidant-response element (ARE), mitogen-activated protein kinases (MAPKs) and caspases by major green tea polyphenol components during cell survival and death. Arch Pharm Res 2000;23:605–12.
[32] Yu R, Chen C, Mo YY, Hebbar V, Owuor ED, Tan TH, Kong A-NT. Activation of mitogen-activated protein kinase pathways induces antioxidant response element-mediated gene expression via Nrf2-dependent mechanism. J Biol Chem 2000;275:39907–13.
[33] Yu R, Mandlekar S, Lei W, Fahl WE, Tan TH, Kong A-NT. p38 mitogen-activated protein kinase negatively regulates the induction of phase II drug-metabolizing enzymes that detoxify carcinogens. J Biol Chem 2000;275:2322–7.
[34] Venugopal R, Jaiswal AK. 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 USA 1996;93:14960–5.
[35] Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 1997;236:313–22.
[36] Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, Yamamoto M. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev 1999;13:76–86.
[37] Chan K, Kan YW. Nrf2 is essential for protection against acute pulmonary injury in mice. Proc Natl Acad Sci USA 1999;96:12731–6.
[38] Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. Science 1998;281:1305–8.
[39] Buttke TM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. Immunol Today 1994;15:7–10.
[40] Slater A, Stefan C, Nobel I, Van Den Dobbelsteen DJ, Orrenius S. Intracellular redox changes during apoptosis. Cell Death Differ 1996; 3:57–62.
[41] Briehl MM, Baker AF. Modulation of the antioxidant defense as a factor in apoptosis. Cell Death Differ 1996;3:63–70.
[42] Busciglio J, Yankner BA. Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro. Nature 1995;378:776–9.
[43] Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng TI, Jones DP, Wang CX. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. Science 1997;275:1129–32.
[44] Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis[see comments]. Science 1997;275:1132–6.
[45] Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang CX. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 1997;91:479–89.
[46] Green DR, Reed JC. Mitochondria and apoptosis. Science 1998;281: 1309–12.
[47] Sen S, M DI. Apoptosis. Biochemical events and relevance to cancer chemotherapy. FEBS Lett 1992;307:122–7.
[48] Manna SK, Mukhopadhyay A, Van NT, Aggarwal BB. Silymarin suppresses TNF-induced activation of NF-kappa B, c-jun N-terminal kinase, and apoptosis. J Immunol 1999;163:6800–9.
[49] Richter M, Ebermann R, Marian B. Quercetin-induced apoptosis in colorectal tumor cells: possible role of EGF receptor signaling. Nutr Cancer 1999;34:88–99.
[50] Simizu S, Takada M, Umezawa K, Imoto M. Requirement of caspase-3(-like) protease-mediated hydrogen peroxide production for apoptosis induced by various anticancer drugs. J Biol Chem 1998;273: 26900–7.
[51] Pagliacci MC, Smacchia M, Migliorati G, Grignani F, Riccardi C, Nicoletti I. Growth-inhibitory effects of the natural phyto-oestrogen genistein in MCF-7 human breast cancer cells. Eur J Cancer 1994;30: 1675–82.
[52] Lippman SM, Shin DM, Lee JJ, Batsakis JG, Lotan R, Tainsky MA, Hittelman WN, Hong WK. p53 and retinoid chemoprevention of oral carcinogenesis. Cancer Res 1995;55:16–9.
[53] Samaha HS, Kelloff GJ, Steele VE, Rao CV, Reddy BS. 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 1997;57:1301–5.
[54] Lepley DM, Pelling JC. Induction of p21/WAF1 and G1 cell-cycle arrest by the chemopreventive agent apigenin. Mol Carcinog 1997;19: 74–82.
[55] Reddy BS, Wang CX, Samaha HS, Lubet R, Steele VE, Kelloff GJ, Rao CV. Chemoprevention of colon carcinogenesis by dietary perillyl alcohol. Cancer Res 1997;57:420–5.
[56] Huang C, Ma WY, Goranson A, Dong Z. Resveratrol suppresses cell transformation and induces apoptosis through a p53-dependent pathway. Carcinogenesis 1999;20:237–42.
[57] Yang GY, Liao J, Kim K, Yurkow EJ, Yang CS. Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. Carcinogenesis 1998;19:611–6.
[58] Yu R, Mandlekar S, Harvey KJ, Ucker DS, Kong A-NT. Chemopreventive isothiocyanates induce apoptosis and caspase-3-like protease activity. Cancer Res 1998;58:402–8.
[59] Mandlekar S, Kong A-NT. Molecular mechanisms of butylated hydroxylanisole-induced toxicity: induction of apoptosis through direct release of cytochrome c. Mol Pharmacol 2000;58:431–7.
[60] Yu R, Jiao JJ, Duh JL, Tan TH, Kong A-NT. Phenethyl isothiocyanate, a natural chemopreventive agent, activates c-jun N-terminal kinase 1. Cancer Res 1996;56:2954–9.
[61] Kirlin WG, Cai J, DeLong MJ, Patten EJ, Jones DP. Dietary compounds that induce cancer preventive phase 2 enzymes activate apoptosis at comparable doses in HT29 colon carcinoma cells. J Nutr 1999;129:1827–35.
[62] Mandlekar S, Yu R, Tan TH, Kong A-NT. Activation of caspase-3 and c-jun NH2-terminal kinase-1 signaling pathways in tamoxifen-induced apoptosis in human breast cancer cells. Cancer Res 2000;60: 5995–6000.
[63] Mandlekar S, Hebbar V, Christov K, Kong A-NT. Pharmacodynamics of tamoxifen and its 4-hydroxy and N-desmethyl metabolites: activation of caspases and induction of apoptosis in rat mammary carcinoma and in human breast cancer cell lines. Cancer Res 2000;60:6601–6.
[64] Clayson DB, Iverson F, Nera EA, Lok E. The significance of induced forestomach tumors. Annu Rev Pharmacol Toxicol 1990;30:441–63.
[65] Mizutani T, Nomura H, Nakanishi K, Fujita S. Hepatotoxicity of butylated hydroxytoluene and its analogs in mice depleted of hepatic glutathione. Toxicol Appl Pharmacol 1987;87:166–76.
[66] Yu R, Hebbar V, Kim D, Mandlekar S, Pezzuto J, Kong A-NT. Resveratrol inhibits phorbol ester and UV-induced AP-1 activation by interfering with tyrosine kinase and mitogen-activated protein kinase pathways. Mol Pharm 2000;60:217–24.