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REDOX REGULATION BY INTRINSIC SPECIES AND EXTRINSIC NUTRIENTS IN NORMAL AND CANCER CELLS

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■ **Abstract** Cells in multicellular organisms are exposed to both endogenous oxidative stresses generated metabolically and to oxidative stresses that originate from neighboring cells and from other tissues. To protect themselves from oxidative stress, cells are equipped with reducing buffer systems (glutathione/GSH and thioredoxin/thioredoxin reductase) and have developed several enzymatic mechanisms against oxidants that include catalase, superoxide dismutase, and glutathione peroxidase. Other major extrinsic defenses (from the diet) include ascorbic acid, β-carotene and other carotenoids, and selenium. Recent evidence indicates that in addition to their antioxidant function, several of these redox species and systems are involved in regulation of biological processes, including cellular signaling, transcription factor activity, and apoptosis in normal and cancer cells. The survival and overall well-being of the cell is dependent upon the balance between the activity and the intracellular levels of these antioxidants as well as their interaction with various regulatory factors, including Ref-1, nuclear factor-κB, and activating protein-1.

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INTRODUCTION

Reactive oxygen species (ROS), such as H_2O_2, superoxide anion ($O_2^{\bullet}$) and the hydroxyl radical ($^{\bullet}OH$), and anion (OH^-) are generated in cells by several pathways and have been implicated in a multitude of physiological processes including aging and immune function, and in disease initiation and progression, such as atherosclerosis and carcinogenesis. Earlier research on free radicals hypothesized that oxidative stress was the primary mechanism by which reactive oxygen species (ROS) influenced biological processes. An elevated oxidized state within a cell can be extremely harmful, resulting in radical generation that leads to lipid peroxidation, DNA cross-linking, and formation of disulfide bonds in proteins. Increased ROS production caused by exposure to carcinogens may result in human cancer and other degenerative diseases. In response to protection against elevated levels of oxidative stress and/or ROS, cells possess several intrinsic systems as well as extrinsic derived antioxidants/reductants, which maintain the intracellular environment in a highly reduced state (130). The major intrinsic systems emphasized in this review include glutathione/oxidized glutathione (GSH/GSSG) and thioredoxin reductase/thioredoxin (TR/TRX), while the extrinsic molecules (among many antioxidants) include vitamin C, carotenoids, and selenium. Other antioxidant enzyme species such as superoxide dismutase (SOD) and catalase also protect the cell against oxidative stress (111).

The study of redox-dependent regulation of molecular processes has gained attention recently (130). In addition to protecting against oxidative stress, research conducted primarily during the past decade has produced evidence of additional

major biological functions of these intrinsic and extrinsic species, including regulation of transcription factors, complex cellular signaling pathways, and cell survival.

BIOCHEMISTRY OF REDOX METABOLISM

Redox Intrinsic Species/Systems

GSH/GSSG One of the major indicators of redox status in the cell is the GSH/GSSG system (159). GSH is a tripeptide of γ-glutamate, cysteine, and glycine and is the predominant low-molecular-weight intracellular thiol, maintained at high concentrations (1–10 mM) in the physiologic state. It is one of the major reductants found in eukaryotic cells. Because the normal cellular state exists primarily in favor of GSH, the intracellular environment is highly reducing, and under physiological conditions, ratios (GSH/GSSG) of 30:1 to 100:1 have been measured (77). The reduced status of the cell in this range protects the cell from oxidants produced during normal metabolism and makes possible reductive biosynthesis (159) (Figure 1).

Glutathione has several biological functions and forms (214). The various forms of the glutathione peroxidase enzymes, a selenium-dependent system, are involved in inactivating ROS, including H_2O_2 and other peroxides, via conversion of the GSH molecule to its oxidized form GSSG. Also, GSH in conjunction with glutathione-s-transferase interacts with various oxidized metabolites, including those produced by estrogen, melanin, prostaglandin, and leukotriene synthesis, as well as with xenobiotics in which GSH forms mercapturates.

TR/TRX The TR/TRX system is a ubiquitous redox couple endogenous within prokaryotic and eukaryotic cells (159). TR is an NADPH-dependent flavin adenine dinucleotide-containing flavoenzyme, and along with TRX plays a key role in maintaining a reduced intracellular environment (7). Interconversion between the oxidized and reduced form of TR requires transfer of electrons from NADPH. The flavin adenine dinucleotide prosthetic group of TR donates the electrons from NADPH to active-site cysteines, resulting in the catalytically active form of TR, which has the ability to reduce the active-site cystine bond of oxidized TRX (Figure 1). Gallegos and colleagues (55) have shown that selenium specifically increases the activity of TR, including stabilizing TRmRNA, which in turn leads to increased TR production and increased growth of normal and cancer cells.

OTHER INTRINSIC REDOX SPECIES Superoxide dismutase, a very important intrinsic enzyme, catalyzes the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2. Several forms of SOD are present in the human cell, including cytosolic Cu/Zn-SOD, mitochondrial SOD, and extracellular SOD (2) (Figure 1).

Hydrogen peroxide in animals is detoxified by catalase as well as by other intrinsic species (111). The enzymatic reaction involves the conversion of hydrogen

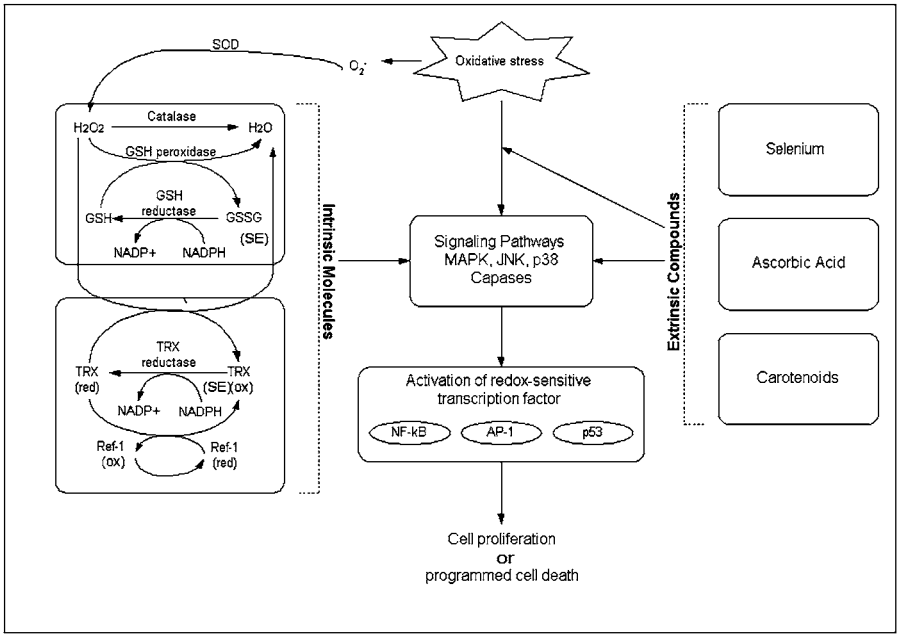

Figure 1 Redox regulation of cellular processes by intrinsic and extrinsic molecules. Various intrinsic redox systems, such as glutathione (GSH/GSSG) and thioredoxin and thioredoxin reductase (TRX/TR), regulate biological processes. Oxidative stress such as ultraviolet radiation results in formation of H_2O_2 and superoxides ($O_2^{\bullet}$). Superoxide dismutase (SOD) catalyzes the conversion of $O_2^{\bullet}$ to H_2O_2, which eventually is converted to water by GSH as well as by TRX. Both TRX and GSH reactions are selenium dependent. Extrinsic molecules, including selenium, ascorbic acid, and carotenoids, also affect cellular processes, including cell signaling, activation of transcription factors, and apoptosis. JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; SE, selenium.

peroxide generated in normal cells to H_2O water by catalase. Even though catalase is not essential for some cell types under normal conditions, this enzyme plays a critical role in the acquisition of tolerance to oxidative stress.

Extrinsic Redox Species and Systems: Focus on Ascorbic Acid, Carotenoids, and Selenium

ASCORBIC ACID The antioxidant activity of vitamin C, primarily via its ability to donate electrons and therefore function as a reducing agent, has been studied extensively (80, 142). The primary dietary sources of vitamin C are fruits and vegetables (67). Specifically, dietary foods rich in vitamin C include grapefruit, honeydew, kiwi, mango, orange, papaya, strawberries, tangerine, and watermelon (67). The antioxidant mechanism of vitamin C occurs via the reduction of highly reactive

radicals, such as hydroxyl, peroxyl, and superoxide radicals, as well as singlet oxygen and reactive peroxides, which can be internally generated via metabolic processes and externally derived from ultraviolet (UV) light and gamma-ray radiation (89). During this process, the relatively unreactive ascorbyl radical is formed (80). Many in vitro and in vivo studies have investigated the antioxidant function of ascorbic acid, which may reduce DNA damage (48, 142, 169, 181) and possibly reduce cancer risk.

CAROTENOIDS The antioxidant action of carotenoids, which protects the cell against oxidative stress (derived from everyday exposure to UV radiation and/or chemicals in the environment), occurs via several mechanisms. Dietary availability of carotenoids is from vegetables and fruits. Carrots are excellent sources of β- and α-carotene, tomatoes are rich in lycopene, dark green leafy vegetables are high in lutein, and orange fruits such as tangerines are rich in β-cryptoxanthin (33).

One of the main mechanisms of the antioxidant action of carotenoids is the ability to quench singlet oxygen ($^1O_2$) (50). Singlet oxygen, the electronically excited form of oxygen, is formed as a result of many reactions in the body, including reactions involving H_2O_2 and peroxide enzymes, but the primary source is exposure to UV light (89). Singlet oxygen is highly reactive and produces cellular damage by oxidizing amino acids found in proteins and nucleic acids, which may result in DNA strand breakage. Carotenoids (CAR, below) can quench these singlet oxygen species by reacting with them, and because of the long-chain hydrocarbon structure of carotenoids, these compounds can return to ground state by releasing a small amount of heat. Through this reaction, the highly reactive singlet oxygen species is also returned to ground state, becoming more stable.

$$^1O_2 + CAR \rightarrow {}^3O_2 + {}^3CAR$$

$$^3CAR \rightarrow CAR + \text{heat}$$

An additional antioxidant action of carotenoids is the ability to interact with free radicals, such as peroxyl or alkoxyl radicals. Carotenoids are capable of transferring electrons (acting as electron donors) and reducing free radicals to nonradical compounds, and in the process produce carotenoid radical cations (98). Thus, suboptimal intake of carotenoids may result in increased DNA damage and contribute to carcinogenesis via reduced action of these antioxidants on singlet oxygen quenching and free radicals.

SELENIUM Selenium has many biochemical roles, including antioxidant functions and regulation of thyroid metabolism, redox status, and immunity (95). Dairy, eggs, fish, meat, grains, and Brazil nuts are rich sources of selenium. Because selenium content and availability in food is highly dependent on the soil in the region, dietary selenium in food varies geographically (33).

Selenium, in the form of selenocysteine, is a major constituent of many antioxidant enzymes known as selenoproteins. Between 30 and 40 selenoproteins have

been identified, and 21 have been further characterized by purification and cloning (114). Two of the selenoproteins identified, GPX and TR (described above), are intricately involved in intracellular redox regulation. The selenoproteins that have been characterized contain selenium at the active site, and therefore many redox reactions/functions are selenium dependent. In addition to regulation of biological processes via selenoproteins, selenium, as reviewed previously, also has direct effects on prevention of oxidative damage, gene expression, and apoptosis (95, 114).

REDOX REGULATION OF BIOLOGICAL PROCESSES IN NORMAL CELLS BY INTRINSIC SPECIES

Cellular Signaling

Cellular signaling involves many cytoplasmic protein kinase cascades (i.e., MAPKs, PI3K/AKT, PKCs) that form pathways connecting exogenous stimuli to the nucleus, resulting in transcription and synthesis of proteins. Cellular signaling activities and expression are very sensitive to both exogenous and intracellular redox status and respond to many exogenous pro-oxidative or oxidative stresses. Many studies demonstrate that multiple intracellular signaling pathways are activated by UV radiation (37, 58, 179, 180, 205), ionizing radiation (9), and heavy metals (105) with generation of ROS, or directly by H_2O_2 (93) and nitric oxide exposure (101), subsequently leading to the activation of nuclear transcription factors in normal cells.

GSH, TRX AND CELLULAR SIGNALING Cellular signaling is regulated by intracellular redox status maintained by reducing antioxidants and redox proteins. Intracellular GSH depletion by DL-buthionine-(S,R)-sulfoximine (BSO), a potent and specific inhibitor of gamma-glutamyl cysteine synthetase, leads to a decline in the levels of GSH. This is followed by an increase in ROS production in a dose- and time-dependent manner with the activation of extracellular signal–regulated kinases (ERK) 1/2 pathway (36). More specifically, protein kinase C (PKC), which has two pairs of zinc finger structures in its regulatory domain and several cysteines in its catalytic sites, has been shown to be sensitive to redox regulation (60). A study by Chu et al. (30) directly demonstrated that regulation of PKC is redox dependent. Purified, recombinant human PKC isoforms were differentially regulated by GSH or oxidized glutathione (GSSG), and cystine-induced inactivation of PKCγ and PKCϵ was rescued by exogenous dithiothreitol and thioredoxin. However, Ward et al. (206) observed an irreversible inactivation of PKC by GSH, suggesting that GSH depletion–mediated PKC activation might be due to the rescue of PKC from inhibition as well as through the induction of novel PKC isoforms and enhanced specific isoform activity (38, 39). Moreover, thioredoxin restored the catalytic activity of PKC-zeta in pulmonary artery endothelial cells in a thiol-reducing dependent mechanism (87). In addition, overexpression of manganese

superoxide dismutase, an antioxidative enzyme, selectively inhibited PKC epsilon and prevented subsequent activation of c-Jun N-terminal kinase (JNK), thereby leading to delayed AP-1 transcriptional activation (222).

Cellular signaling such as PKC activation also influences intracellular redox status by affecting the expression or activity of certain redox-regulating proteins. The activity of TRX and TR in normal epidermis extracts was increased significantly with application of the PKC activator 12-O-tetradecanoylphorbol-13-acetate (TPA) (99), while Anema et al. (3) reported downregulation of TR expression via transiently exposing cells to TPA in cultured human umbilical-vein endothelial cells. In addition, TPA treatment increased PKC phosphorylation of the apurinic/apyrimidinic endonuclease 1 (APE/Ref-1) protein (75). APE/Ref-1 regulates the redox status of many transcription factors. Another study, conducted by Flaherty et al. (49), found that TPA in conjunction with granulocyte-macrophage colony-stimulating factor increased Ref-1 protein levels, but TPA alone produced no increase.

TRX, GSH, and Regulation of Transcription Factors

A growing body of literature suggests that regulation of gene expression by redox-sensitive transcription factors is dependent on the redox status of the cell (56). Two of the intrinsic redox elements discussed above (TRX and GSH), as well as extrinsic nutrients (vitamin C, carotenoids, and selenium), are involved in regulating the actions of various transcription factors, including nuclear factor-κB (NF-κB) and activator protein-1 (AP-1). The regulation of these transcription factors is complex. Studies have shown that under different conditions both antioxidants and oxidants activate AP-1 (121). In normal cells, both transcription factors induce gene regulation that influences inflammation, immune function, cell growth and survival, and stress responses (89).

TRX and GSH may have overlapping as well as compartmentalized functions in activation and regulation of transcription factors, including regulation of NF-κB and AP-1 in the cytoplasm and the nucleus (66, 71). Earlier studies showed that exogenous treatment or transient expression of TRX resulted in a dose-dependent downregulation/inhibition of NF-κB, while AP-1 activity was strongly upregulated and enhanced (121, 178). Galter and colleagues (56) showed that both NF-κB and AP-1 were activated upon treatment of cells with 1,3-bis-(2-chloroethyl)-1-nitrosourea, a compound that, among other functions, elevates intracellular levels of GSSG by inhibiting glutathione reductase. Gel shift assays and transfection studies with reporter constructs also showed that GSSG inhibited DNA-binding ability of NF-κB and AP-1. A more recent study provides evidence that TRX has a differential role in regulating NF-κB in the cytoplasm and nucleus (71). TRX inhibited NF-κB activity in the cytoplasm, while upregulating the ability of NF-κB to bind to DNA in the nucleus. Other studies have shown that TRX can also regulate several other transcriptional factors, including hypoxia-inducible factor-1 (HIF-1), Nrf-2, and cytochrome P450's (66, 76, 209). Hansen et al. (66) assessed

the role of TRX and GSH in Nrf-2-dependent signaling. Nuclear translocation (which measures cytoplasmic activity) of Nrf-2 was regulated via GSH, whereas nuclear activity was primarily controlled by TRX.

Regulation of Apoptosis

ROS AND ANTIOXIDANTS IN APOPTOSIS Mitochondria are the specialized organelles that are involved in the control of apoptosis via production of ROS. Many studies have indicated a role for ROS in the regulation of apoptosis. This subject has been reviewed extensively (19, 64, 204). Generally, apoptosis can be induced by the addition of ROS such as H_2O_2 (63) or by depletion of cellular exogenous antioxidants such as GSH (6, 21, 31). In other instances, apoptosis is associated with stimulation and production of intracellular ROS (43, 224). It is reasonable to postulate that antioxidants might prevent apoptosis through a redox-based mechanism. Both intracellular and exogenous antioxidants such as GSH (188) and thioredoxin-1 (10) as well as N-acetylcysteine (176) protect cells from apoptosis or the activation of apoptosis-related signaling pathway. Furthermore, cells overexpressing the antioxidant enzyme SOD are relatively resistant to radiation-induced apoptosis (211). However, other studies have also revealed that the apoptosis response was conserved in mitochondrial DNA experimentally depleted cells, which were deficient in respiration; this finding suggests that mitochondrial oxidative phosphorylation is not essential or only attenuates related signals for apoptosis in certain cases (81, 85).

TRX AND REGULATION OF APOPTOSIS The role of the TRX/TR pathway in apoptosis has been investigated. Exogenous thioredoxin-1 prevented apoptosis of lymphoid cells induced by glutathione depletion (79) or of apoptosis in human neuroblastoma SH-SY5Y cells induced by a Parkinsonian-producing neurotoxin (2). Adding TRX to culture media significantly prolonged the survival of B-cell chronic lymphocytic leukemia cells, and release of an autocrine growth factor was observed, which suggests an increased cell survival (135). Overexpression of thioredoxin-1 protected cells not only from oxidative stress-induced apoptosis (137) but also from cytotoxic and DNA-damaging effects of many chemotherapeutic drugs such as cisplatin, mitomycin C, doxorubicin, etoposide, staurosporine, thapsigargin, and diepoxybutane (10, 174, 218). The mechanisms of action might be related to its regulation of NF-κB or AP-1 cell survival pathways (52, 112), as well as inhibition of tumor-suppressor protein PTEN (120) and proapoptotic proteins such as apoptosis signal-regulating kinase 1 (ASK1) (8, 175), which binds to thioredoxin directly in a redox-dependent manner and results in the loss of their kinase activities.

Mitochondria thioredoxin (thioredoxin-2, TRX-2) is a critical antioxidant in regulating mitochondrial ROS-induced cytotoxicity. TRX-2-deficient cells showed an accumulation of intracellular ROS and induced cytochrome c release into the cytosol, followed by mitochondria-dependent apoptosis (195). Similarly, Zhang

et al. (221) demonstrated that TRX-2 and TRX-1 cooperatively inhibited ASK1 activities. Overexpression of TRX-2 in endothelial cells blocked ASK1-induced apoptosis as well as oxidant-induced apoptosis in human osteosarcoma cells (27). However, Patenaude et al. (152) reported that the cellular responses to various prooxidant or nonoxidant apoptotic stimuli were quite similar between mitochondrial thioredoxin reductase-2 (TRX R2) or TRX-2 overexpressed cells and controls. The use of different cell lines in the respective studies may explain the divergence of these conclusions.

GSH AND REGULATION OF APOPTOSIS The protective role of GSH against apoptosis and cell death emanates from multifactorial mechanisms that involve detoxification and modulation of cellular redox state and the subsequent redox-sensitive cell-signaling pathways (115, 220), as well as the interaction with pro- and antiapoptotic signals. Upon withdrawal of interleukin-3, cells overexpressing Bcl-xL (antiapoptosis member) failed to lose significant amounts of GSH and no apoptosis was evident, while GSH in Bax (proapoptosis member) overexpressing cells was significantly reduced and sensitive to apoptosis, indicating a possible role of GSH in Bcl-xL-mediated antiapoptosis mechanisms (15). Additionally, GSH depletion by BSO treatment significantly enhanced Bax-induced apoptosis in four non-small-cell lung cancer cell lines (74), suggesting that the redox status maintained by thiols may interfere with Bax-mediated apoptosis. Increased GSH levels were observed in melphalan-induced apoptosis in human melanoma cells by inhibition of Bax/cytochrome c redistribution (12). More importantly, increasing evidence has shown an intimate link between GSH and antiapoptotic Bcl-2 and Bcl-2-mediated apoptosis. The cells overexpressing Bcl-2 had significantly higher levels of GSH with translocation into the nucleus (200), but the increase in GSH content was not due to increased synthesis (119). Consistently, depletion of GSH within Bcl-2 high-expression cells resensitized them to apoptosis without alternating Bcl-2 protein levels (124, 213). GSH also prevented apoptosis through the inhibition of AP24, an apoptotic protease that transmits signals to induce DNA fragmentation (213). Therefore, GSH levels are an important factor in the efficacy of anticancer drug–induced apoptosis. It is well documented that depletion of GSH facilitates tumor treatment, with higher response to apoptosis induction.

REF-1 AND APOPTOSIS The human DNA repair enzyme APE/Ref-1 is a dual function protein that plays an important role in both DNA base excision repair and in transcriptional responses to oxidative stress (45, 53). Endogenous AP sites are produced through a variety of mechanisms, i.e., oxidative damage, resulting in mutations or genetic instability. APE/Ref-1 is one of the key enzymes involved in the repair process. Moreover, in addition to its DNA repair activity, APE/Ref-1 has also been found to facilitate the DNA-binding activity of several transcription factors, including AP-1, NF-κB, Myb, p53, and Pax, through both redox-dependent and redox-independent mechanisms.

The relationship of Ref-1 and apoptosis has been extensively studied. This protein has a dual function with overlapping endonuclease and redox properties. Many studies have revealed that upregulation of Ref-1 protects cells from various apoptosis stimuli, including oxidative stress, chemotherapeutic drugs, and radiation treatment, as well as tumor necrosis factor (TNF) superfamily receptor 6 (Fas)-mediated cell death signal (4, 61, 62, 170). In contrast, downregulation of Ref-1 expression is associated with apoptosis and sensitization of cells to induced apoptosis both in vitro (46, 171) and in vivo (91, 128). Most importantly, a cause-and-effect relationship between apoptosis and Ref-1 levels was first reported by Robertson et al. (171), who found that when apoptosis was blocked by Bcl-2 (anti-apoptosis member) overexpression, reduction of Ref-1 was no longer evident and cells underwent differentiation. Consistently, preventing the loss of Ref-1 by inhibiting protein synthesis rescued neurons from experimentally induced cell death (28). The possible mechanisms involved might be related to redox-activation by APE/Ref-1 of many cell survival signals, such as NF-κB and AP-1, and maintenance of genomic integrity through enhanced repair of cytotoxic DNA lesions (167), through serving as an adaptive response to apoptotic stimuli. However, Gaiddon et al. (54) reported that coexpression of Ref-1 potentiated p53-induced apoptosis in H1299 cells, and supported the conclusion drawn by Yao et al. (217) who showed a possible relationship between Ref-1 induction and the occurrence of hypoxia-induced apoptosis. The role of Ref-1 in contributing to apoptosis may depend on the different types of cell lines and the nature of the underlying stresses used for such studies.

MITOCHONDRIAL REDOX

Mitochondrial DNA (mtDNA) codes for 13 respiratory chain subunits and exhibits high susceptibility to mutation compared with nuclear genomic DNA (110). The presence of mtDNA mutations in many cancer cells has been demonstrated, including in solid tumors of breast, colon, stomach, liver, kidney, bladder, head/neck, and lung, as well as for hematologic diseases such as leukemia, myelodysplastic syndrome, and lymphoma [reviewed in depth by Copeland et al. (32) and Penta et al. (154)]. Wei & Lee (208) demonstrated that impairment in mitochondrial respiration and oxidative phosphorylation elicits an increase in oxidative stress that causes a host of mtDNA rearrangements and deletions. Vergani et al. (202) also found that mitochondrial DNA depletion resulted in an oxidized intracellular redox status. In addition, Carew et al. (23) reported that in human chronic lymphocytic leukemia, mtDNA mutations appeared to be associated with increased ROS generation. Patients who were refractory to conventional therapeutic agents tended to have higher mutation rates than did patients who responded to treatment. These observations suggest that normal mitochondrial function is not only essential for cellular biological activities but also is crucial for maintaining intracellular redox status.

Mitochondria are the most redox-active organelles. Maintaining mitochondrial redox status depends not only on total cellular redox environment but also on its own reduction capacities, including glutathione pools, antioxidant enzymes, and mitochondrial thioredoxins such as TRX-2 (177). Alterations in mitochondrial redox status significantly affected the apoptotic process. Experimentally induced GSH deficiency in newborn rats has been shown to result in striking enlargement and degeneration of mitochondria (82). Increasing evidence also has demonstrated that upregulation of mitochondrial antioxidant proteins such as TRX-2 and peroxiredoxin-3 (Prdx3) effectively protected cells from mitochondrial-mediated apoptosis (27, 136, 221). An antiapoptotic phenotype has been observed in drug-resistant cells with mitochondrial morphological alterations (42).

REDOX REGULATION OF BIOLOGICAL PROCESSES IN NORMAL CELLS BY EXTRINSIC NUTRIENT SPECIES

Vitamin C

VITAMIN C AND TRANSCRIPTION FACTORS The action of ascorbic acid (AA) on redox-dependent biological function is due to the direct redox role of AA and its metabolites as well as to generation of the ascorbate free radical. Recently, in vitro and in vivo studies have demonstrated the ability of AA to regulate factors that may influence gene expression, apoptosis, and other cellular functions (22, 24, 108, 125, 133, 160, 168). Carcamo and coworkers (22) loaded cells with AA and then induced oxidative stress, which generated dehydroascorbic acid. NF-κB binding was suppressed while IKB kinase beta was unaffected, which suggests that vitamin C plays a role to down-modulate cell signaling (Table 1).

Several studies investigated the mechanism by which ascorbic acid regulates the AP-1 complex, including the Fos (c-Fos, Fos B, and Fra-1) and Jun (c-Jun, Jun B, and Jun D) superfamilies (5, 25, 26) (Table 1). Catani and coworkers (26) examined the role of ascorbate in the regulation of several transcripts, including GST-pi, MLH1, and fra-1 (a member of the FOS family of transcription factors that regulate AP-1). After loading of vitamin C in normal human keratinocytes, fra-1 messenger RNA was induced within two hours. The same group showed that application of ascorbate inhibits expression of c-Jun and c-Fos, which are involved in AP-1 activity. Furthermore, after exposure to UV-B radiation, ascorbate-treated cells led to a 50% decrease in JNK phosphorylation (which activated AP-1), thus inhibiting the JNK/AP-1 signaling pathways.

Studies in healthy subjects have shown that supplementation with vitamin C can reduce oxidative protein damage and modulate expression of adhesion molecules, such as ICAM, which via various signaling pathways may be involved in carcinogenesis and atherosclerosis (24, 168, 212). One study supplemented 40 healthy subjects (half of the subjects had low plasma ascorbate levels) with 250 mg/day of ascorbate and measured modulation of ICAM-1 expression (168). Prior to

TABLE 1 Nutrients and their role in the regulation of biological processes*

Nutrient	Regulation of biological intermediates
Ascorbic acid	Regulation of transcription factors/transcripts NF-κB AP-1, fra-1 gene, Fos (c-Fos, Fos B, Fra-1) and Jun (c-Jun, Jun B and Jun D) superfamilies GST-pi MLH1 Regulation of apoptosis Fas induced Adhesion molecules, ICAM IL-1 downregulation
Carotenoids (β-carotene, α-carotene lutein, lycopene, β-cryptoxanthin)	Regulation of transcription factors Via RAR, RXR NF-κB Apoptosis Proinflammatory cytokines, IL-6, TNF-alpha BCL-2 Bax
Selenium	Regulation of transcription factors NF-kB AP-1 P53 Cell signaling intermediates MAPK, JNK, ERK p38 Regulation of apoptosis BCL-2 Bax

*Abbreviations: AP-1, activating protein-1; Bax, important proapoptosis mediator; BCL-2, an important antiapoptosis mediators; ERK, extracellular-signal-regulated kinase; Fas, TNF superfamily receptor 6; GST, glutathione-S-transferase; IL-1, interleukin-6; JNK, c-Jun amino-terminal kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappa-B; RAR, retinoic acid receptor; RXR, retinoid X receptor; TNF, tumor necrosis factor.

supplementation, subjects with low plasma ascorbate had higher levels of ICAM-1 mRNA, and after supplementation for six weeks, ICAM-1 mRNA was significantly reduced in those with low plasma levels. The results suggest that antioxidant supplementation can influence cellular response, including gene expression and cell signaling.

VITAMIN C AND APOPTOSIS In most studies, vitamin C, the major water-soluble antioxidant, inhibits cell death triggered by various stimuli, and much of this protection has been attributed to its antioxidant property (20, 98, 109, 155, 156, 193). Studies of the antiapoptotic activity of vitamin C in monocytes and thymocytes have provided evidence of a role of vitamin C in modulation of the immune

system (109, 155). A recent study by Perez-Cruz et al. (155) investigated the role of vitamin C and FAS-induced apoptosis (155). Fresh human monocytes as well as U937 cells (of monocytic origin) were incubated with vitamin C. Accumulation of vitamin C inhibited FAS-mediated cell death, and apoptosis was associated with reduced levels of ROS; decrease in activity of capase 3, 8, and 10; and preservation of mitochondrial membrane integrity.

Studies using mouse models/cells as well as humans also have demonstrated the protective effect of ascorbate against UV damage or other stimuli promoting cell death (20, 156, 193, 198). Stewart and coworkers (193) showed that mouse keratinocytes preincubated with ascorbic acid exhibited a significant decrease in DNA adducts induced by UVB radiation. Another study tested whether mouse T-cells incubated with ascorbic acid reduced T-cell apoptosis (20). Both activated and resting T-cells responded to ascorbic acid, and T-cell apoptotic pathways induced by three different conditions were inhibited, including growth factor withdrawal and spontaneous and steroid-induced death, which suggests that vitamin C may protect the immune system from overproliferation via this mechanism. In human keratinocytes, cells incubated with ascorbate showed a substantial decrease in apoptosis induced by UV radiation; this protection by ascorbic acid was related to reduced lipid peroxidation as well as downregulation of proinflammatory cytokines, such as IL-1 (198). Therefore, in vitro and in vivo data suggest that vitamin C protects against cell death in cells exposed to UV-mediated cytotoxicity.

Carotenoids

CAROTENOIDS AND TRANSCRIPTION FACTORS Emerging evidence suggests that carotenoids or their derivatives, including retinoic acid (RA), interact and regulate transcription factors (134). It has been well established that retinoic acid, the parent compound of retinoids, produces multiple effects on cells, including inhibition of cell proliferation and enhanced cell differentiation. Via its interaction with two nuclear receptors—retinoic acid receptors and retinoid X receptors—RA regulates biological processes. The binding of the retinoic acid ligand to these receptors, located in the nucleus of the cell, mediates upregulation or inhibition of gene expression. Recently, studies have assessed whether lycopene may interact as a ligand with nuclear receptors and have found that the action of lycopene and other carotenoids either was similar to RA or substantially weaker (185).

CAROTENOIDS AND APOPTOSIS Studies assessing carotenoids and their effects on regulation of transcription factors and apoptosis have primarily investigated β-carotene. Tests conducted in acinar pancreatic cells from mice showed that cells under oxidative stress upregulated NF-κB and increased production of inflammatory cytokines (183). However, supplementation with β-carotene suppressed NF-κB activation and production of interleukin-6 (IL-6) and TNF-alpha inflammatory cytokines (184), which suggests that β-carotene has a protective effect. Additional in vivo studies reveal that normal cells supplemented with carotenoids

are protected from programmed cell death (159, 161, 162). Prior to exposure to single-dose UVB radiation, Skh-1 mice were administered lutein plus zeaxanthin for two weeks (59). Supplementing with these carotenoids produced a significant decrease ($p < 0.05$) in proliferating cells and proliferating cell antigen. Also, signal transduction intermediates JNK and p-JNK were inhibited and antiapoptotic protein BCL-2 was upregulated by β-carotene supplementation, while the apoptotic BAX protein was downregulated after induction by external stimuli, which suggests that carotenoids appropriately attenuate apoptosis in normal cells (161, 162).

Selenium

SELENIUM AND TRANSCRIPTION FACTORS A majority of the action and regulation of transcription factors by selenium occurs via selenoproteins, including TR and GPX. Selenium as a constituent of TR can regulate DNA-binding ability of NF-κB and AP-1 as well as other transcription factors.

Selenite and selenodigulathione are effective oxidants of reduced thioredoxin and reduced TR, which diminishes the TR pool and may explain the inhibitory influence of selenium on cell growth and cancer progression (192). After incubation of lymphocytes with selenite and selenodigulathione, a 50% inhibition of AP-1 DNA binding was observed, which suggests a mechanism for the anticancer influence of selenium. Another study reported similar results, showing that selenite blocked AP-1 DNA binding by 50% and decreased expression of an AP-1-dependent gene (65). A more recent study in human leukocytes showed that selenomethionine and selenocysteine inhibited nuclear accumulation of AP-1 and NF-κB elicited by an exogenous oxidant (86).

Selenium has been shown to influence NF-κB activity (18, 97, 98, 109). The role of selenium in the regulation of NF-κB activity is supported by data showing that treatment of cell cultures with selenium alone and/or overexpression of selenodependent GPX reduce activation of NF-κB (18, 97, 98, 109). In human cells overexpressing GPX, exposure to H_2O_2 resulted in a reduction of intracellular ROS accumulation and decreased NF-κB activity (97).

Selenium and selenoproteins have also been shown to regulate p53 (131, 153, 189). It is well established that the tumor suppressor protein p53 has a multitude of cellular functions, from gene regulation to DNA repair. Several functions of p53 are redox regulated (150, 153). The ability of p53 to induce transcription was measured in yeast; p53 activity was suppressed in yeast deficient for the gene encoding TR (153). Furthermore, results showed that p53 functioned as a transcription factor only after reduction of disulfides, a finding that suggests activity of p53 is selenium dependent as well as redox related (153).

SELENIUM AND CELLULAR SIGNALING Selenium is involved in signal transduction via its activation of several intermediates, including mitogen-activated protein kinases (MAPKs) and transcription factors such as AP-1 and NF-κB (as discussed above), which influence gene expression and cell growth. Park and coworkers (149)

assessed the molecular mechanism of selenium in cell signaling, and found that selenite inhibits both JNK and p38, which are subfamilies of the MAPK signaling pathway. In vitro studies showed that selenite directly regulated JNK, and upon addition of reductants, the inhibition of JNK by selenite was reversed, which suggests that a thiol redox mechanism was involved in the repression of JNK by selenite. Another report also found that ebselen, a seleno-organic compound, inhibited nitric oxide and NF-κB by suppressing phosphorylation of JNK; however, p38 was not modulated (186). These results suggest that selenium can regulate NF-κB and protooncogene expression via an effect on signal transduction intermediates. A more recent in vitro experiment demonstrated that selenium suppressed the p38 MAPK pathway and prevented lipopolysaccharide inflammatory response (94). Therefore, modulation of signaling cascades by selenium affects various cellular activities, including cell survival, apoptosis, and gene expression. In vivo studies are needed to confirm the importance of these observations.

SELENIUM AND APOPTOSIS A large body of evidence has shown that selenium is involved in the molecular processes leading to cell death. However, the role of selenium in apoptosis is complex because of its pro-oxidant as well as antioxidant functions (114). In normal cells, the activity of selenium as an antioxidant confers protection against cell death via regulation of cell-signaling molecules (166, 219). A study supplementing keratinocytes with 50 nM of selenomethionine conferred 95% protection against cell death induced by 960 J/m^2 UVB, while 100% of cells were rescued from apoptosis against 600 J/m^2 UVB (166). Recent studies from the same group further indicated that incubation of keratinocytes with selenomethionine and selenite protected cells against oxidative DNA damage, and supplementation of cells with selenocompounds reduced cellular apoptosis initiated by UV radiation by 71% (164, 165). Selenium also protected cells from UV radiation–induced apoptosis; however, selenium had minimal influence on p53 expression, which suggests that selenium protection against apoptosis is independent of the p53 pathway and may impact cellular survival via regulation of the Fas/Fas ligand or TNF-α pathways (164). In addition, it has been demonstrated (as shown above) that TRX and GPX—both selenoproteins—are involved in apoptosis; therefore, selenium as a component of these proteins indirectly affects apoptosis.

REDOX ABERRATIONS IN CANCER CELLS

General Aberrations

Much evidence has shown that redox balance is impaired in cancer cells compared with normal human cells (138, 194), which may be related to oncogenic stimulation (11) and/or mitochondrial malfunction. Altered levels of antioxidative enzymes (i.e., SOD, catalase, and glutathione peroxidase) and nonenzymic antioxidants (i.e., GSH; vitamins A, C, and E; thioredoxins; and Ref-1), as well as the

related signal pathways, are evident in many human tumors and are fundamentally involved in carcinogenesis and tumor progression (Figure 2).

Elevated levels of mRNA thioredoxin-1 have been reported and increased protein levels have been observed in many human primary tumors, including cervical cancer, non-small-cell lung cancer, pancreatic cancer, and hepatoma (extensively reviewed in 203). In addition, Lincoln et al. (104) found that in aggressive invasive mammary carcinomas and advanced malignant melanomas, TRX expression was substantially elevated compared with less aggressive tumors. TRX expression in neoplastic cells was also found in both the nucleus and cytoplasm of the neoplastic cells and was positively correlated with TR levels and localization. These observations suggested that increased TRX/TR regulates both nuclear and cytoplasmic redox status as well as activates nuclear transcription factors, facilitating a more aggressive potential for tumor cells. Elevated TRX-1 not only contributes to the development of tumor resistance (215, 218) but also is closely associated with patient outcome. A recent study of primary non-small-cell lung cancer found an association among TRX-1, regional lymph node involvement, and decreased patient survival (88). A study reported by Raffel et al. (163) also demonstrated a significant association between increased TRX-1 expression and colorectal cancer patient survival.

It is well documented that Ref-1 is involved in different stages of tumor development (initiation, promotion, and progression) through maintenance of intracellular redox status and activation of a cell survival signal, as well as through repair of damaged DNA lesions. Many tumors, including cervical, pediatric rhabdomyosarcomas, prostate, epithelial ovarian cancers, and germ cell, exhibit elevated Ref-1 expression in comparison with normal tissues (45). Recently, Orii et al. (141) also reported that a higher predominance of Ref-1 active-form expression was observed in almost all leiomyoma and leiomyosarcoma samples compared with that of normal myometrial cells; this might be related to the higher proliferative activity and could contribute to the selective growth advantage of these tumors. Additionally, as Ref-1 exhibits both cytoplasmic and nuclear enzymic activities, specific subcellular localization of Ref-1 has also been investigated in many human tumors. Epithelial ovarian cancers display both nuclear and cytoplasmic staining, with cytoplasmic localization predominating, whereas normal tissue exhibited strong nuclear staining without cytoplasmic staining (126). Tanner et al. (196) reported elevated nuclear APE/Ref-1 expression during the progression of ovarian carcinomas. Importantly, Ref-1 expression in tumor cells also was closely related to treatment resistance. Robertson et al. (170) demonstrated that higher levels of APE/Ref-1, observed in testicular cancer of various histologies, might be associated with their relative resistance to bleomycin and radiation. Hedley and collaborators (69) found highly significant increases in thioredoxin and Ref-1 expression within the hypoxic microenvironment of invasive cervical carcinomas. Herring et al. (70) also found that there was a correlation between intrinsic radiosensitivity and expression of Ref-1 in cervical carcinomas. Because cells exposed to hypoxia in tissue are resistant to radiation and are associated with poor prognosis (73), this observation leads to the

conclusion that elevated thioredoxin and Ref-1 may act in a coordinated manner to promote cancer cell survival.

GSH, GSH-related Enzymes in Cancer Cells

Gamma-glutamyl cysteine synthetase (GCS), the rate-limiting enzyme in GSH biosynthesis, is highly expressed in most cases of malignant mesothelioma and inhibition of GCS by BSO potentiated cisplatin-induced cytotoxicity (83). In human colorectal carcinomas, strong cytoplasmic staining for GCS heavy subunit was detected with a higher frequency than in adenoma and was significantly correlated with multidrug-resistance protein 1 expression (197). Another GSH-related enzyme, glutathione-S-transferase (GST), was elevated in colonic neoplasia compared with adjacent normal mucosa (129), but only a marginal increase in breast cancer was noted compared with normal adjacent tissues (44). Nelson et al. (132) also observed that loss of GSTP1 expression in prostate cancer appeared to be characteristic for prostatic epithelial neoplasia lesions, and was most often associated with somatic "CpG island" DNA methylation changes. In human glioma cell lines, high levels of GST-pi protein expression correlated with enhanced sensitivity to vincristine-induced cell death (210).

Melanoma

The skin is chronically exposed to both endogenous and environmental pro-oxidant agents such as UV radiation, a well-known initiator and promoter of skin cancers (16). The imbalance between pro-oxidant and antioxidant activities in skin cells subsequently leads to ROS-mediated oxidative damage and might contribute to skin diseases (17). Our group (122) has intensively investigated the abnormal redox status in human melanoma compared with normal melanocytes. Melanin acts not only as an antioxidant to neutralize ROS (187) but also as a pro-oxidant (47, 191). We have proposed that continuously elevated intracellular ROS is caused, at least in part, by the presence of oxidized melanin functioning as a pro-oxidant that subsequently results in dysregulated nuclear transcription signals, including NF-κB (116, 117, 122) and AP-1 (216), leading to the transcription of genes related to melanoma tumorigenesis, promotion, and progression.

DIETARY NUTRIENTS AND INFLUENCE ON CANCER CELLS

Carotenoids

Several investigations have demonstrated the antiproliferative effect of carotenoids on various cancer cell lines (1, 14, 107, 151, 190). Inhibition of cell cycle progression by lycopene has been shown in breast, lung, and prostate cell lines (90, 107, 151). Human prostate cancer cell lines were incubated with either lycopene

alone or with lycopene plus α-tocopherol (151). The combination of the two antioxidants strongly inhibited prostate cancer cell proliferation, whereas lycopene alone had very little effect. In contrast, lycopene alone was shown to regulate transcription factors (90). MCF7 mammary cancer cells supplemented with lycopene significantly reduced the insulin-like growth factor-I induction of cell signaling and inhibited AP-1 binding; these results suggest that lycopene has an inhibitory effect on mammary cancer cell growth.

Attenuation of apoptosis by carotenoids in cancer cells has been documented previously (144). Palozza and colleagues showed a proapoptotic effect of β-carotene in different cancer cell lines (including human colon and leukemic cancer cell lines), but the cell lines had varying degree of sensitivity to β-carotene (143, 145, 146). The same group investigated the role of β-carotene in attenuation of apoptosis via regulation of NF-κB in colon and leukemic cancer cell lines (148). The findings clearly showed that β-carotene, via a redox mechanism, increased ROS and GSSG/GSH ratio, and these results were associated with increased NF-κB binding ability, inhibition of cell growth, and enhanced proapoptotic activity in tumor cells (147). Cleavage products of β-carotene were also shown to regulate breast cancer cell proliferation, the effects of which may be mediated by regulation of AP-1 (199). Another study showed that carotenoids, by interacting with cell membrane molecules, activated the capase-8 cell-signaling pathway, which was pivotal for initiating apoptosis, in human colon, leukemic, and melanoma tumor cell lines (146). In congruence with these results, β-carotene has been shown to inhibit the expression of the antiapoptotic protein Bcl-2 in cancer cells, thereby reducing growth of cancer cells (144). These data suggest that carotenoids regulate proliferation and growth as well as apoptosis via attenuation of various transcription factors and signaling intermediates.

Selenium

From epidemiological to clinical to basic science data, selenium has been shown to have anticarcinogenic properties. Several studies have demonstrated the antigrowth effect of selenium in androgen-sensitive LnCAP cells as well as in androgen-resistant lines such as DU-145 and PC3 (118, 201, 207). Numerous mechanisms have been delineated for the anticarcinogenesis function of selenium, including antioxidant function, regulation of cell signaling, and proapoptotic influence in cancer cells (95).

Premalignant human breast cell lines were incubated with methylselenic acid, a form of selenium (40). Both cell lines exhibited growth inhibition and induction of apoptosis. Methylselenic acid altered the expression of 30 genes, which were categorized into cell-cycle regulators (include cyclin A and D1, p16, and p27) and apoptotic and signaling genes, such as MAPK and ERK (kinases) (40). Additional reports have suggested that the molecular basis for selenium in inducing apoptosis in cancer cells occurs via mediation of cell signaling targets, including caspase 8 and 9 pathways (41, 57, 78, 84).

The influence of selenium on molecular processes including cell signaling and apoptosis has been shown to occur (but not exclusively) via a redox-dependent mechanism (183, 223). Regulation of p53 by selenomethionine required redox-dependent Ref-1 (183). After sustained exposure to selenite, upregulation of redox-sensitive proteins, manganese superoxide dismutase and p21, was observed (223). These data suggest that selenium, in different forms, attenuates various targets and regulates apoptosis and cell signaling.

Other Dietary Compounds (Resveratrol, Epigallocatechin-3-Gallate, Isothiocynates)

Although the current review emphasizes carotenoids, selenium, and vitamin C, there is growing evidence that other dietary constituents, including resveratrol, epigallocatechin-3-gallate (EGCG), and isothiocynates function as chemopreventive agents and modulate cellular processes in cancer cells (13, 92, 100). Resveratrol is found in many plant species, including grapes, and therefore is a component of red wine (13). Resveratrol induces cell cycle arrest and apoptosis in many cancer cell lines. The proapoptotic action of resveratrol is associated with p53 and JNK activation. EGCG, a component of green and black tea, also exerts differential effects on cancer and normal cells, where cancer cells are more responsive to EGCG (202). Human colorectal cells treated with EGCG experienced cell death and exhibited nuclear condensation, DNA fragmentation, and caspase activation. Both resveratrol and EGCG also have been shown to affect NF-κB activation. Also, cruciferous vegetables, such as cabbage, broccoli, and cauliflower, are high in isothiocynates and demonstrate chemopreventive function (92). The anticarcinogenic function of isothiocynates can partially be explained via the upregulation of phase II detoxifying enzymes and inhibition of phase I carcinogen-activating enzymes by isothiocynates. Isothiocynates also induce cell cycle arrest and modulate NF-κB and AP-1, which elicit an apoptotic response in cancer cells.

POTENTIAL FOR INTERVENTION IN REDOX SYSTEMS AND SPECIES

Molecular Manipulation

Normally, topical application or oral administration of antioxidants to scavenge ROS or depletion of antioxidants to potentiate ROS damage are methods used to enhance, respectively, the preventive or chemotherapeutic effect (17, 115). Recently, extensive studies on the role of TRX, GSH, and Ref-1 in promotion, progression, and drug resistance have confirmed their potential as attractive targets for the development of new cancer-preventive and therapeutic strategies (45, 115, 158).

DRUGS THAT INTERACT WITH GSH IMPAIR INTRACELLULAR REDOX BUFFERING SYSTEM It is well established that elevated GSH renders cancer cells resistant

against chemotherapy and radiation treatment; therefore, this compound might be a good candidate target for further manipulation. Many compounds that interfere with the GSH pathway through different mechanisms have exhibited promising antitumor activities, either by inducing apoptosis or by reversing drug resistance to chemotherapeutic agents in tumor cells.

BSO, which induces GSH depletion through inhibiting the synthesis of γ-glutamylcysteine, sensitizes HL-60 human leukemia cells as well as arsenic-resistant acute promyelocytic leukemia-derived cell lines to arsenic trioxide treatment (34, 35), and also restores the senescence of resistant tumor cells to melphalan both in vitro and in vivo (140, 182, 200). Cepsilon, isolated from the stem bark of *Magnolia sieboldii*, induces apoptosis in human promonocytic leukemia U937 cells by rapidly depleting the intracellular GSH and protein thiols (29). The growth-inhibitory effect of allicin, the major ingredient of crushed garlic, was also correlated with GSH levels (72). TER199, an analog of glutathione designed to be a specific inhibitor of GST P1-1, has also significantly reversed the multidrug-resistance protein 1-mediated drug resistance for vincristine, doxorubicin, etoposide, and mitoxantrone (139). TER286, a novel nitrogen mustard alkylating prodrug activated by GST, has selectively exhibited more toxicity to tumors expressing higher GST P1-1 levels (173).

DRUGS THAT INHIBIT TRX Elevated levels of TRX or TR have been found in many aggressive human tumors that exhibit a lower apoptosis rate, a higher growth potential, and an elevated invasion capacity, implicating the involvement of TRX/TR in different stages of tumor development (104). Based on the observations that the lower TRX content in SH-SY5Y neuroblastoma cells causes higher susceptibility to serum deprivation–induced apoptosis (2) and that TRX antisense effectively reduced the anchorage-independent potential of Smad7-overexpressing cells (8), it has been suggested that specific TRX inhibitors might exhibit inhibitory effects on cancer cell growth. 1-Methyl-propyl-2-imidazolozyl disulfide (PX-12), a TRX-1 inhibitor binding to the Cys73 residue, not only significantly potentiated the inhibitory effect of cisplatin on pancreatic cancer (8) but also exhibited promising antitumor activities among a variety of tumor cell lines both in vitro and in vivo (158).

DRUGS THAT INHIBIT REF-1 Ref-1 seems to be a central protein that regulates the action of many transcription factors and provides a unique link with oxidative stress, redox-activated transcription factors, and DNA base excision repair (45). The elevated Ref-1 in many cancers is always associated with aggressive proliferation, increased resistance to therapeutic agents and poor prognosis (as stated in the "Redox Aberrations in Cancer Cells" section above). Decreasing Ref-1 inhibited platelet-derived growth factor–induced proliferation as well as cell cycle progression from G0/G1 to S phase (68). In addition, Lau et al. (103) found that APE/Ref-1 antisense significantly potentiated the cytotoxity of gemcitabine in Panc-1 cells. These observations have supported further investigation of novel strategies for

cancer treatments that target Ref-1, either by enhancing the sensitivity of tumor cells to chemotherapy or by inhibiting proliferation. By using sophisticated computer programs to model the three-dimensional structure of Ref-1/APE protein and virtual screening software to dock molecules from virtual drug libraries to the protein, our group has found several compounds that inhibit the endonuclease activity of Ref-1 in vitro (unpublished data). Preliminary data also showed that some of these compounds exhibit active antimelanoma activities and sensitize melanoma cells to dacarbazine treatment.

DIETARY INTERVENTION TRIALS AND FUTURE DIRECTION

Observational epidemiology studies have suggested that the consumption of fruits and vegetables and/or micronutrients, including carotenoids, vitamin E, selenium, folate, and polyphenols, is related to a reduction in cancer risk (51, 113). Nonetheless, case-control and cohort studies are subject to confounding and other biases; therefore, several randomized controlled trials have been conducted to test the effects of dietary supplementation with micronutrients on cancer risk (51). The Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Trial assessed the influence of supplementation with β-carotene and α-tocopherol on lung cancer risk in Finnish male smokers. The results of the ATBC trial showed that supplementation with β-carotene and α-tocopherol slightly increased the incidence of lung cancer in smokers. A subanalysis revealed that this increased risk was strongly associated with heavy smoking and drinking. Additional research conducted by the Beta-Carotene and Retinol Efficacy Trial, which tested a combination of β-carotene and retinol, showed a 28% increased risk in lung cancer in the group supplemented with β-carotene and retinol (51). Possibly the doses used in these studies were too high. Recent studies have also shown the pro-oxidant influence of high amounts of β-carotene in the presence of cigarette smoke in a ferret model (103, 106). Also, it may well be that β-carotene is not the only constituent in vegetables and fruits that confers anticarcinogenic effects.

Randomized clinical trials also have been conducted to assess the influence of fat, fiber, vegetable, and fruit intake on cancer risk (51). Several trials, including the Polyp Prevention Trial, investigated the effects of consuming a high-fiber, low-fat, high-vegetable and -fruit diet on adenomatous polyp recurrence. The trials followed participants for one to four years. Little to no significant reduction in polyp recurrence was observed. Possible contributors to the null results include a small sample size, insufficient follow-up time, and wrong endpoints, as well as the failure to assess other protective dietary sources and micronutrients in the trial. Currently, an intervention trial enrolling 3000 women is examining the influence of an overall dietary pattern of high fruit and vegetable intakes on breast cancer recurrence (157, 172).

The question of whether diet or dietary nutrients can reduce incidence of cancer and/or function in a chemopreventive manner has been the subject of much discussion. The limitations of randomized dietary clinical trials in which interventions were conducted with only a single nutrient are considerable. Supplementation with a single nutrient in prevention trials overlooks the pivotal impact that other micronutrients collectively assert. Many nutritional compounds and redox systems influence regulation of biological processes governing cellular signaling, transcription factor activity, and apoptosis, which are all intricately involved in determining whether a cell progresses from a normal cell to a cancer cell. However, because a number of extrinsic and intrinsic redox species regulate different and/or the same molecular targets, designing intervention studies that can effectively incorporate these numerous variables is complex. Nonetheless, randomized clinical trials are still the gold standard in investigating further the results derived from observational and laboratory studies on the association between nutrition and cancer prevention and control. Also, chemoprevention strategies and randomized clinical trials may require modulation/interventions of overall dietary patterns, such as increasing vegetable, fruit, and fiber intake and/or a "cocktail" of micronutrients working together in concert to introduce a sustained protection against cancer risk outcomes (123).

In addition, identifying appropriate molecular markers and targets is critical in the discovery of new therapies to reduce cancer risks. It is well established that TRX, Ref-1, and GSH do not act in isolation, but form an intricate network in the cell; therefore, blockade of one target alone might not be sufficient to achieve a clinical benefit. Thus, multitargeted compounds or targeted compounds used in combination likely are of more powerful and promising potential for cancer prevention and treatment, both with respect to efficacy and for prevention of resistance. Also, screening natural compounds or dietary components targeting these signals would shed new light on cancer chemoprevention, with high efficacy and low toxicity.

Redox regulation involves intrinsic components, including TRX, GSH, and extrinsic micronutrients, including selenium, vitamin C and carotenoids, which function in distinct, yet additive, synergistic and/or antagonist roles to control cellular processes. Because a multitude of molecules govern the same and/or different pathways, to move the field of redox regulation forward, expertise in various fields is required. Therefore, future clinical trials will need to include a multidisciplinary approach and include molecular biologists, nutritionists, biochemists, epidemiologists and clinicians to translate redox regulation of biological processes at the cellular level to humans in efforts to achieve the greatest public health benefit.

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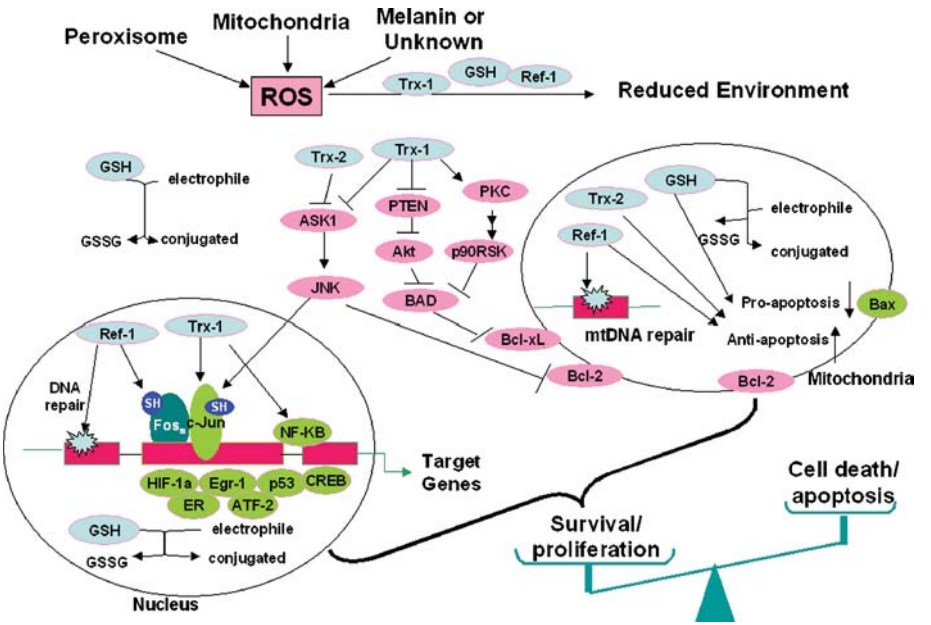

Figure 2 Abnormal redox-mediated signal regulation in cancer cells. The cumulative production of reactive oxygen species found in cancer cells is associated with altered redox regulation of signaling. In certain compartments, especially the nucleus and mitochondria, elevated levels of thioredoxin and apurinic/apyrimidinic endonuclease 1 as well as glutathione contributed to a reducing environment. This intracellular milieu not only facilitates escape from apoptosis but also produces an aggressive proliferation potential through activation of cell survival signals mediated by redox-sensitive nuclear transcription factors such as AP-1 and NF-κB, as well as by interfering with the cellular apoptosis pathway, both directly and indirectly. ASK1, apoptosis signal-regulating kinase 1; JNK, Jun N-terminal kinase; Akt, AKT8 virus oncogene cellular homolog; PTEN, phosphatase and tensin homologue deleted on chromosome 10; ER, estrogen receptor; HIF-1α, hypoxia inducible factor-1α; ATF-2, activating transcription factor-2; Egr-1, early growth response-1; CREB, cAMP response element-binding protein.

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