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THE ROLE OF OXIDATIVE STRESS IN CARCINOGENESIS

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■ **Abstract** Chemical carcinogenesis follows a multistep process involving both mutation and increased cell proliferation. Oxidative stress can occur through overproduction of reactive oxygen and nitrogen species through either endogenous or exogenous insults. Important to carcinogenesis, the unregulated or prolonged production of cellular oxidants has been linked to mutation (induced by oxidant-induced DNA damage), as well as modification of gene expression. In particular, signal transduction pathways, including AP-1 and NFκB, are known to be activated by reactive oxygen species, and they lead to the transcription of genes involved in cell growth regulatory pathways. This review examines the evidence of cellular oxidants' involvement in the carcinogenesis process, and focuses on the mechanisms for production, cellular damage produced, and the role of signaling cascades by reactive oxygen species.

OVERVIEW

Chemically induced neoplasia is a multistep process involving DNA damage and cell proliferation. Chemical carcinogens impact on various stages of this process and function through modification of cellular and molecular events. Investigators recognizing the apparent differences by which chemicals participate in the carcinogenesis process, proposed the use of the descriptors, "genotoxic" and "epigenetic" (nongenotoxic), to help further refine the mechanisms by which a carcinogen was functioning (1). Genotoxic agents usually refer to chemicals that directly damage genomic DNA, which in turn can result in mutation and/or clastogenic changes. Chemicals in this category are frequently activated in the target cell and produce a dose-dependent increase in neoplasm formation. A second category of carcinogenic compounds (nongenotoxic) appear to function through non-DNA reactive or indirect DNA reactive mechanisms. Although much less is known about the exact mode of action of nongenotoxic carcinogens, they modulate cell growth and cell death. Changes in gene expression and cell growth parameters are paramount in the action of nongenotoxic carcinogens. These agents frequently function during the promotion stage of the cancer process (2, 3).

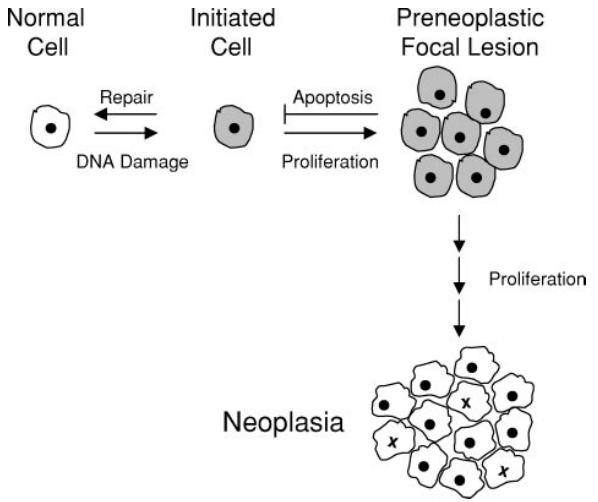

Figure 1 The stages of the carcinogenesis process. Initiation involves a nonlethal mutation in DNA that produces an altered cell followed by at least one round of DNA synthesis to "fix" the genetic damage produced by initiation. The promotion stage is characterized by the clonal expansion of initiated cells by the induction of cell proliferation and/or inhibition of apoptosis, resulting in the formation of an identifiable focal lesion. The promotion stage requires the continuous presence of the promoting stimuli, and thus it is a reversible process. Progression is the final stage in the carcinogenesis process. This stage is characterized by accumulation of additional genetic damage, leading to the transition of cells from a benign to maglignant phenotype, and is considered an irreversible process.

The induction of neoplasia in rodents by chemical and physical agents involves a multistage process. At least three distinct stages of the carcinogenesis process have been defined (2). These include initiation, promotion, and progression (Figure 1). Initiation involves the formation of a mutated, preneoplastic cell from a genotoxic event. The formation of the preneoplastic, initiated cell is an irreversible, but dose-dependent process. Promotion involves the selective clonal expansion of the initiated cell through an increase in cell growth through either an increase in cell proliferation and/or a decrease in apoptosis in the target cell population (4). The events of this stage are dose dependent and reversible upon removal of the tumor promotion stimulus. Progression, the third stage, involves cellular and molecular changes that occur from the preneoplastic to the neoplastic state. This stage is irreversible, involves genetic instability, changes in nuclear ploidy, and disruption of chromosome integrity.

Increased replicative DNA synthesis and subsequent cell division is important in each of the stages of carcinogenesis (2, 5). Two possible mechanisms have been proposed for the induction of cancer. In one, an increase in DNA synthesis

and mitosis by a nongenotoxic carcinogen may induce mutations in dividing cells through misrepair. With continual cell division, mutations will result in an initiated preneoplastic cell that may clonally expand to a neoplasm. In addition, nongenotoxic agents may serve to stimulate the selective clonal growth of already "spontaneously initiated cells" (6).

In maintaining cell number within a tissue, an equilibrium exists between cell proliferation and cell death. The cancer process thus is a result of an imbalance between cell growth and death. Endogenous and exogenous factors that influence DNA damage, cell growth, and cell death contribute to carcinogenesis. Experimental evidence supports an important role for reactive oxygen species in the cancer process. Increases in reactive oxygen in the cell, through either physiological modification or through chemical carcinogen exposure, contribute to the carcinogenesis processes. This may be via genotoxic effects resulting in oxidative DNA adducts or through modification of gene expression.

SOURCES OF REACTIVE OXYGEN SPECIES

A substantial body of evidence has been produced that links the production of reactive oxygen radicals, and subsequently oxidative stress and damage, to the pathogenesis of age-related and chronic diseases including cancer (7–10). Oxidative stress has been defined as an imbalance between oxidants and antioxidants in favor of the former, resulting in an overall increase in cellular levels of reactive oxygen species (11). Reactive oxygen species can be produced by both endogenous and exogenous sources. Potential endogenous sources include oxidative phosphorylation, P450 metabolism, peroxisomes, and inflammatory cell activation (Table 1).

During mitochondrial oxidative metabolism, the majority of the oxygen consumed is reduced to water; however, an estimated 4% to 5% of molecular oxygen is converted to reactive oxygen species, primarily superoxide anion, formed by an initial one-electron reduction of molecular oxygen (Table 2). Superoxide can be dismutated by superoxide dismutase to yield hydrogen peroxide (12). In the presence of partially reduced metal ions, in particular iron, hydrogen peroxide is subsequently converted through Fenton and Haber-Weiss reactions to a hydroxyl radical (13). The hydroxyl radical is highly reactive and can interact with nucleic acids, lipids, and proteins (13).

Neutrophils, eosinophils, and macrophages are an additional endogenous source and are major contributors to the cellular reactive oxygen species. Activated macrophages, through "respiratory burst," elicit a rapid but transient increase in oxygen uptake that gives rise to a variety of reactive oxygen species, including superoxide anion, hydrogen peroxide, and nitric oxide (Table 1). In addition, peroxynitrite is formed from the coupling of nitric oxide and superoxide (9, 10; Table 2). The release of the biologically active molecules (e.g., cytokines and reactive oxygen intermediates), from activated Kupffer cells (the resident macrophage of the liver), has been implicated in hepatotoxicological and hepatocarcinogenic events (14, 15). More recently it has been demonstrated that the Kupffer cell may

TABLE 1 Reactive oxygen and nitrogen species generation and removal in the cell

Cellular oxidants	Source	Oxidative species
Endogenous	Mitochondria	$O_2^{-\bullet}$, H_2O_2, $^\bullet OH$
	Cytochrome P450	$O_2^{-\bullet}$, H_2O_2
	Macrophage/inflammatory cells	$O_2^{-\bullet}$, $^\bullet NO$, H_2O_2,OCl^-
	Peroxisomes	H_2O_2
Exogenous	Redox cycling compounds	$O_2^{-\bullet}$
	Metals (Fenton reaction)	$^\bullet OH$
	Radiation	$^\bullet OH$

Cellular antioxidants

Enzymatic	Nonenzymatic
Superoxide dismutase	Vitamin E
Catalase	Glutathione
Glutathione peroxidase	Vitamin C
Glutaredoxin	Catechins
Thioredoxin	

Oxidants > Antioxidants → Oxidative damage (DNA, RNA, lipid, protein)

Oxidants can be produced via both endogenous and exogenous sources. Antioxidants function to maintain the cellular redox balancing. However, excess production of oxidants and/or inadequate supplies of antioxidants results in damage to cellular biomolecules and may impact on neoplastic development.

TABLE 2 Pathways for intercellular oxidant generation

1. Generation of reactive oxygen species via reduction of molecular oxygen
   $O_2 + e^- \rightarrow O_2^{-\bullet}$ (superoxide anion)
   $O_2^{-\bullet} + H_2O \rightarrow HO_2^\bullet$ (hydroperoxyl radical)
   $HO_2^\bullet + e^- + H \rightarrow H_2O_2$ (hydrogen peroxide)
   $H_2O_2 + e^- \rightarrow OH^- + {}^\bullet OH$ (hydroxyl radical)
2. Production of reactive nitrogen species
   L-ARGININE + $O_2 \rightarrow {}^\bullet NO$ (nitric oxide) + L-CITRULLINE
   $O_2^{-\bullet} + {}^\bullet NO \rightarrow ONOO^-$ (peroxynitrite)
   $ONOO^- + CO2 \rightarrow ONOOCO_2^-$ (nitrosoperoxy carbonate)
   $ONOOCO_2^- \rightarrow {}^\bullet NO_2$ (nitrogen dioxide) + $CO_3^{-\bullet}$ (carbonate anion radical)
3. Fenton reaction
   $H_2O_2 + Fe^{2+} \rightarrow OH^- + {}^\bullet OH + Fe^{3+}$

1. A series of oxygen radicals are produced by the reduction of molecular oxygen. Of the radicals produced, the hydroxyl radical, hydroperoxyl radical, and the superoxide anion are sufficiently reactive and may interact with biomolecules.
2. Through nitric oxide synthase, nitric oxide is produced from reaction of molecular oxygen and L-ARGININE. Following the production of this radical, and interaction with superoxide anion, a number of oxidizing and reactive species are produced.
3. Fenton reaction can produce the hydroxyl radical. Transition metals such as Cu(2+), Cr(V), and Ni can also catalyze this reaction and result in radical formation.

participate at the tumor promotion stage of carcinogenesis. Activation of Kupffer cells with LPS resulted in an increase in focal volume and DNA synthesis within diethylnitrosamine-induced hepatic foci, whereas inactivation of Kupffer cells using dietary glycine ablated the LPS-induced effects on liver cell growth (16, 17). These results provide evidence linking the products released from the activated Kupffer cell to the tumor promotion stage of the carcinogenesis process.

Metabolic activation and production of reactive oxygen species by cytochrome P450 has been proposed by Parke and associates (18). Reactive oxygen species can be produced from several sources during metabolism, including (*a*) through redox cycling in the presence of molecular oxygen, (*b*) through peroxidase-catalyzed single-electron drug oxidations, and (*c*) through "futile cycling" of cytochrome P450 (18, 19). Through the induction of cytochrome P450 enzymes, the possibility for the production of reactive oxygen species, in particular, superoxide anion and hydrogen peroxide, arises following the breakdown or uncoupling of the P450 catalytic cycle (19). P450 2E1 is involved in the oxygenation of substrates such as ethanol, and is capable of generating a prolonged burst of reactive oxygen species near the site of substrate oxidation (20). Similarly, metabolism of phenobarbital by P4502B results in the uncoupling of the catalytic and subsequent release of superoxide anion (21). Thus, the correlation between induction of P450 isozymes and the subsequent reactive oxygen species production warrants consideration as a possible mechanism for the induction of oxidative stress and tumor promotion seen following exposure to a number of chlorinated and nonchlorinated compounds such as dieldrin, TCDD, lindane, and phenobarbital (22).

The potential for the production of oxidative species derived from peroxisomes has also been proposed. Chemicals such as peroxisome proliferators are potent inducers of cytochrome P450 4A, and induce the formation of peroxisomes, and as such, an increase in H_2O_2 production. Through peroxisome proliferation and increased peroxisomal enzyme activity, H_2O_2 will escape and shift the cellular redox balance toward the oxidative state (23, 24). Recent studies have suggested that although increases in oxidative DNA damage have been observed with selective peroxisome proliferators, the increases apparently do not correlate with the relative peroxisome proliferating activity or neoplastic responses (14). These latter findings do not preclude a role for oxidative stress in the cancer induction by peroxisome proliferators but may exclude a causal link between the peroxisome proliferation and the cancer process.

Reactive oxygen species can be produced by a host of exogenous processes. Environmental agents including nongenotoxic carcinogens can directly generate or indirectly induce reactive oxygen species in cells (25). The induction of oxidative stress and damage has been observed following exposure to xenobiotics of varied structures and activities. Chlorinated compounds, radiation, metal ions, barbiturates, phorbol esters, and some peroxisome proliferating compounds are among the classes of compounds that have been shown to induce oxidative stress and damage in vitro and in vivo (22). 2-Butoxyethanol is an example of a chemical that produces reactive oxygen species indirectly, resulting in liver cancer

in mice. Reactive oxygen species production, as evidenced by the induction of 8-hydroxyguanosine in liver, appears to result from Kupffer cell activation secondary to 2-butoxyethanol-induced hemolysis and subsequent hepatic iron deposition (26). The role of Kuppfer cell–derived reactive oxygen species and the potential importance of Kupffer cell activation to the carcinogenesis process are intriguing and require further study.

ANTIOXIDANTS

Under normal physiological conditions, cells are capable of counterbalancing the production of reactive oxygen species with antioxidants (Table 1). Endogenous cellular antioxidant defenses are mainly enzymatic and include superoxide dismutase, glutathione peroxidase, and catalase. Superoxide dismutases are localized to the cytosol and mitochondria and function to reduce superoxide anion to hydrogen peroxide and water. Glutathione peroxidases, localized in the cytosol and mitochondria, remove the majority of hydrogen peroxide, whereas catalase, located in peroxisomes, is responsible for the removal of high levels of hydrogen peroxide (13, 27). Nonenzymatic antioxidants such as vitamin E, vitamin C, β-carotene, glutathione, and coenzyme Q function to quench reactive oxygen species (28). When the redox balance is shifted in favor of cellular oxidants, oxidative damage to nucleic acids, lipids, or proteins can result and produce modification to cell function and cell viability. Interestingly, many of the cellular antioxidants are regulated in part by the redox status of the cell.

The intracellular redox state is determined by the relative amount of reduced and oxidized form of each redox pair, and it may determine changes in the cellular redox state by shifting this value toward oxidizing or reducing conditions. Reactive oxygen species therefore are important determinants of the redox state and constitute a regulatory mechanism in the cell through modification of protein conformation and function and regulation of signal transduction (29, 30). The redox systems commonly found within the cells include nicotinamide adenine dinucleotide phosphate, thioredoxin, glutaredoxin, and glutathione. Among these, glutathione is important to the overall cellular redox balance. Because cellular glutathione concentration is $\sim$500- to 1000-fold higher than the other redox regulating proteins, changes in the ratio of reduced to oxidized glutathione are directly reflective of intracellular redox alterations (31).

Glutathione, the most abundant low-molecular-weight thiol in mammalian cells, is present in reduced (GSH) and oxidized (GSSG) forms (32). The reduced form of glutathione is 10- to 100-fold higher than the oxidized form. An increase in intracellular GSSG can arise from the breakdown of H_2O_2 by glutathione peroxidase (33). Because of the relatively low concentration of GSSG in the cell compared with GSH, a minor elevation in the oxidation of GSH to GSSG can result in a significant elevation in intercellular GSSG levels. Oxidized glutathione can be reduced to GSH by the NADPH-dependent glutathione reductase as well as via the thioredoxin/glutaredoxin systems. Glutathione also modulates the activity of

thiol-dependent enzymes that contain cysteine residues sensitive to redox changes (34, 35). GSH also is used as a cofactor for antioxidant enzymes such as GSH peroxidase, involved in the reduction of peroxides, including membrane lipids peroxides formed upon oxidative insults (36).

The GSH/GSSG ratio is normally closely regulated. Disruption of this ratio is involved in several cellular reactions involved in signal transduction and cell cycle regulation under conditions of oxidative stress; the GSH/GSSG ratio tends to decrease either through an increase in the level of glutathione disulphide or a decrease in reduced glutathione (31, 37, 38). The redox balance can be maintained, however, even in the face of an oxidative stress by increasing glutathione reductase activity or via elimination of GSSG from cells (31).

γ-Glutamylcysteine synthase (γ-GCS), an enzyme involved in glutathione synthesis, belongs to a class of proteins that respond to oxidizing conditions as a regulatory mechanism (39). Oxidizing conditions that result in GSH depletion promote a conformational change in γ-GCS that increases the catalytic activity of this enzyme, thus stimulating the synthesis of glutathione, while physiological GSH concentration reduces GSH synthesis through feedback inhibition mechanisms (39). Therefore, it is presumed that decreased GSH would contribute to cell death only when oxidative stress becomes prolonged, and cellular systems are not sufficient to counteract the reactive oxygen species mediated insult.

Mammalian and prokaryotic thioredoxins are proteins with oxidoreductase activity (40). Specific disulphide targets for reduction by thioredoxins include ribonucleotide reductase protein disulphide isomerase and the transcription factors p53, NF-κB, and AP-1 (41, 42). Thioredoxin are involved in the reduction of cellular peroxides (43, 44). In addition, thioredoxin is directly involved in the reduction of reactive oxygen species, refolding of oxidized proteins, and the induction of growth-factor-like effects and cytokine activity. The latter is important in stimulating proliferation and the growth of tumor cells (45).

Glutaredoxin, also known as thioltransferase, is a small (12 kDa) dithiol protein involved in the redox regulation of transcription factors (e.g., NFκB, AP-1) and signaling cascades (46). Glutaredoxin is reduced by glutathione, which is in turn reduced by NADPH and glutathione reductase (47). It has been suggested that glutaredoxin acts as a biological sensor that recognizes conditions of increased GSSG and responds by initiating signal transduction pathways (48).

REDOX STATE AND CARCINOGENESIS

GSH concentrations in the cell have been linked with the induction of apoptosis (49, 50). Upon apoptotic stimulus, cytochrome *c* release from mitochondria occurs in concert with a massive extrusion of GSH through specific membrane translocators (49). This finding appears related to apoptosis because restoring the intracellular GSH content through preventing extrusion blocked the induction of apoptosis (50). However, depletion of intracellular GSH failed to induce apoptosis, which indicates that GSH depletion is not the only factor involved in the

commitment to undergo apoptosis. Although a relationship between intracellular glutathione levels and mitochondria-dependent apoptotic pathway is evident, the target molecules and/or redox-sensitive steps that connect GSH status to the apoptotic pathway have yet to be determined.

Reducing or oxidizing molecules, such as thioredoxin and/or H_2O_2, can be present outside the cell, thus changing the extracellular redox milieu. Furthermore, H_2O_2 can cross the plasma membrane and enter the cells, leading to changes in the intracellular redox environment. In addition, membrane proteins, such as NADPH oxidase on neutrophil membranes, can produce H_2O_2 (51). Cells may produce H_2O_2 as a byproduct of enzyme reactions such as with γ-GT, an essential enzyme for GSH uptake across the plasma (52). In this reaction, H_2O_2 is produced as the peptide bond between glutamic acid and cysteinyl-glycine is cleaved. It has been reported that the antitumor activity exerted by GSH on human ovarian carcinoma cells as well as the prevention of apoptosis and maintenance of proliferation in these cells is mediated by γ-GT-produced H_2O_2 (53, 54). It is clear that both the genesis of cellular oxidants as well as the balance of cellular redox status involves complex regulatory pathways, many of which are still being elucidated. What is equally unequivocal is that cellular oxidants, when left unbalanced by antioxidants and other reducing equivalents, result in oxidative modification to cellular constituents.

OXIDATIVE DNA DAMAGE AND CARCINOGENESIS

As noted above, reactive oxygen species can arise through a variety of events and pathways. In a given cell, an estimated 10^5 oxidative lesions per day are formed (55). Over 100 oxidative DNA adducts have been identified (56–58). Reactive oxygen species can directly produce single- or double-stranded DNA breaks, purine, pyrimidine, or deoxyribose modifications, and DNA cross-links. Persistant DNA damage can result in either arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability, all of which are seen in carcinogenesis.

Many forms of reactive oxygen species are capable of forming oxidized bases. The hydroxyl radical in particular has been shown to produce a number of oxidized DNA lesions (59). The reactivity of the hydroxyl molecule is such that its migration in the cell is limited and thus reacts quickly with cellular components (11). For the hydroxyl radical to react and oxidize DNA, it must be generated adjacent to the nucleic acid material. H_2O_2, a precursor to hydroxyl radical, is less reactive and more readily diffusible and thus more likely to be involved in the formation of oxidized bases (7, 12). Peroxynitrite, another strong cellular oxidant, is formed from the coupling of nitric oxide and superoxide (60, 61). As with H_2O_2, peroxynitrite is diffusible between cells and is taken up by active transport mechanisms into cells (62). Equally important to the induction of mutation by reactive oxygen species is the fact that nitric oxide and superoxide are produced in activated macrophages, and as such, it is likely that peroxynitrite is formed in proximity to these cells. The DNA

damaging capability of peroxynitrite may therefore help to explain the reported association between inflammation and mutation (59).

Oxidation of guanine at the C8 position results in the formation of 8-hydroxydeoxyguanosine (OH8dG), probably the most studied oxidative DNA adduct. This oxidative DNA lesion results in site-specific mutagenesis, is mutagenic in bacterial and mammalian cells, and produces G → T transversions that are widely found in mutated oncogenes and tumor suppressor genes (63–65). In addition, reactive oxygen species can react with dGTP in the nucleotide pool to form OH8dG. Therefore, it is postulated that during DNA replication, OH8dG in the nucleotide pool will be incorporated into DNA opposite dC or dA on the template strand, resulting in A:T to C:G transversions (58, 66). OH8dG also produces dose-related increases in cellular transformation, which can be prevented by antioxidants, further supporting the role of OH8dG in the carcinogenic process (67). Other oxidative DNA lesions, such as 8-oxo-adenine, thymine glycol, 5-hydroxy-deoxycytidine, as well as several uracil analogs, have been shown to be mutagenic (68, 69). In summary, oxidized DNA bases appear to be mutagenic and capable of inducing mutations that are commonly observed in neoplasia.

Reactive nitrogen species, such as peroxynitrites and nitrogen oxides, have also been implicated in cancer formation (70). Upon reaction with guanine, peroxynitrite has been shown to form 8-nitroguanine (71). Due to its structure, this adduct has the potential to induce G:C → T:A transversions (72). The likelihood of this adduct being formed and inducing mutations in vivo is low because this lesion is not stable (71). In RNA, however, this nitrogen adduct is stable (73). Other agents that incorporate into RNA, such as 5-fluorouridine, interfere with RNA metabolism, and a similar effect might be expected with the RNA nitrogen adduct. The pathological consequences of 8-nitroguanine and the potential linkage to the carcinogenesis process are not known.

MITOCHONDRIAL DNA DAMAGE AND CARCINOGENESIS

Although importance has been placed on the role of oxidative nuclear DNA damage in neoplasia, other evidence has demonstrated the involvement of the mitochondrial oxidative DNA damage in the carcinogenesis process (74, 75). Supporting the notion that the mitochondria are under the burden of sustained oxidative stress and increased mutation frequency is the finding that tumor cells appear more glycolytic compared with normal cells (76). The sustained oxidative burden in the mitochondria has been linked to the induction of mutation. Mitochondrial DNA mutations and alterations in mitochondrial genomic function appear to be causally related to the development of neoplasia. Mitochondrial DNA mutations have been identified in a number of cancers (77, 78). Altered expression and/or mutations in mitochondrial genes encoding for complexes I, III, IV, and V, and in the hypervariable regions of mitochondrial DNA, have been identified in human

tumors. Compared with the nuclear genome, the mitochondrial genome appears to be more susceptible to oxidative base damage (79, 80). Although alterations in mitochondrial genes have been found in human tumors, mutagenesis occurs more rapidly in rodents compared with humans (81). Furthermore, the mutation rate in mitochondrial DNA has been reported to be at least two orders of magnitude higher than that of nuclear DNA (81). At least three factors for the increased susceptibility of the mitochondrial genome should be considered. (*a*) Mitochondrial DNA is in close proximity to the electron transport system, a major source of reactive oxygen species. Under physiological conditions, the mitochondria convert 4% to 5% of oxygen consumed into superoxide anion and subsequently hydrogen peroxide (12). (*b*) Mitochondrial DNA is not protected by histones. (*c*) DNA repair capacity is limited in the mitochondria, which completely lack nucleotide excision repair (82, 83). Collectively, these finding may partially explain the increased frequency of mitochondrial mutations seen in tumor cells.

Although the extent to which mitochondrial DNA alterations participate in the cancer process is unknown, significant information exists that supports the involvement of the mitochondria in carcinogenesis. Although the portion of tumor cells that possess mutated mitochondrial DNA has not been fully established, a commonality of specific mutations has been noted. Mutations in genes encoding oxidative phosphorylation can affect cellular ATP production and an overall cellular energy imbalance. Decreases in ATP can affect the cell cycle by blocking progression through the cell cycle (84). Fragments of mitochondrial DNA have been found to be inserted into nuclear DNA. This has been suggested as a mechanism for activation of oncogenes (85). Hydrogen peroxide and other reactive oxygen species have been implicated in the activation of nuclear genes that are involved in mitochondrial biogenesis, transcription, and replication of the mitochondrial genome. Low levels of hydrogen peroxide appear to be stimulatory to mitogenesis in a variety of mammalian cell types (86). The stimulation of mitochondrial biogenesis may be a cellular response to compensate dysfunctional oxidative phosphorylation associated with mutated mitochondrial DNA. As observed with oxidative genomic DNA modification, oxidative damage and the induction of mutation in mitochondrial DNA (and perhaps altered mitochondrial function) may participate at multiple stages of the process of carcinogenesis, involving mitochondria-derived reactive oxygen species, induction of mutations in mitchondrial genes, and perhaps the insertion of mitochondrial genes into nuclear DNA.

REPAIR OF OXIDATIVE DNA DAMAGE

DNA repair enzymes and repair pathways exist that function to remove altered bases produced by oxidative-mediated reactions. Several oxidative DNA repair enzymes have been identified from mammalian cells that function to remove oxidized bases from DNA and nucleotide pools (87). Paradoxically, the efficiency of repair may be enhanced following exposure to reactive oxygen species because expression of many of DNA repair enzymes is upregulated following oxidative stress (88).

In nuclear DNA, approximately 90% of oxidized bases are repaired by single nucleotide repair mechanisms and the remaining 10% by long-patch base excision repair. This indicates that single nucleotide base excision repair is the primary pathway for repair of OH8dG (89). Although significant knowledge of the DNA repair mechanisms in nuclear DNA exists, little is known about the repair systems in the mitochondria. However, compared with nuclear DNA repair mechanisms, in the mitochondrion, DNA repair capacity appears to be low. Insufficient repair capacity may lead to mitochondrial dysfunction and degenerative diseases. Only base excision repair is known to be present in the mitochondria, and furthermore, unlike nuclear base excision repair that has long-, short-patch, and transcription-coupled repair, only short-patch repair has been demonstrated in the mitochondria (90). It is known that OH8dG accumulates in mitochondrial DNA. Interestingly, the activity of mitochondrial OGG1, the major DNA glycosylase involved in the repair of oxidized bases in the mitochondria, increases with age whereas nuclear OGG1 activity exhibits a slight decrease with age (91). This finding suggests that the expression of these two isoforms may be differentially regulated and is consistent with the notion that mitochondrial DNA is more prone to oxidative damage than nuclear DNA.

LIPID DAMAGE AND CARCINOGENESIS

Aside from oxidized nucleic acids, other oxidation derived DNA adducts appear important in chemical carcinogenesis. Radical-mediated damage to cellular biomembranes results in lipid peroxidation, a process that generates a variety of products including reactive electrophiles such as epoxides and aldehydes (92). Malondiadlehyde (MDA), a by-product of lipid degradation, is a tautomer that is both highly electrophilic and nucleophilic. This characteristic allows not only reaction with cellular nucleophiles, but also the formation of MDA oligomers (93). MDA and MDA-MDA dimers are mutagenic in bacterial assays as well as in the mouse lymphoma assay (94). MDA was also shown to induce thyroid tumors in chronically treated rats (95). MDA reacts with several nucleic acid bases to form dG, dA, and dC adducts (96). The identification of MDA-DNA adducts in humans may be significant as MDA-DNA adducts have been detected in the genome of healthy humans in quantities comparable to those levels generated by exogenous chemicals in rodent carcinogenesis studies. The observed MDA-DNA adducts appear to be promutagenic as they induce mutations in oncogenes and tumor suppressor genes seen in human tumors. MDA-DNA adduct levels also apprear to correlate with altered cell cycle control and gene expression in cultured cells (97).

OXIDATIVE STRESS AND CELL GROWTH REGULATION

A role for reactive oxygen species production and oxidative stress has been proposed for both the stimulation of cell proliferation and for cell deletion by apoptosis (98, 99). The mechanisms for the involvement of oxidative stress in the induction

of the cell proliferation and apoptotic processes are not known, but clearly do not involve a universal mechanism. The effects of reactive oxygen species and oxidative stress within cells appear to be cell specific and dependent upon the form as well as the intercellular concentration of reactive oxygen species. Thus, the involvement of reactive oxygen species in cell growth regulation is complex, and dependent on a number of cellular and biochemical parameters.

Reactive oxygen species function to induce cell proliferation during the tumor promotion stage of carcinogenesis (100). Both H_2O_2 and superoxide anion induce mitogenesis and cell proliferation in several mammalian cell types (101). Furthermore, a reduction in cellular oxidants via supplementation with antioxidants such as superoxide dismutase, catalase, β-carotene, and flavenoids inhibits cell proliferation in vitro (102). Oxidative stress also modulates apoptosis. High concentrations of reactive oxygen species trigger an apoptotic signaling pathway, resulting in cell loss (103). A number of endogenous substances (prostaglandins, and lipid hydroperoxides), redox cycling compounds (quinones, adriamycin), and growth factors (transforming growth factor β and tumor necrosis factor α) induce apoptosis via the generation of reactive oxygen species (104, 105). Antioxidants such as *N*-acetyl cysteine (NAC), glutathione, and dithiothreitol inhibit the apoptotic process, further supporting the link between reactive oxygen species induction and apoptosis (105). Xenobiotics may differentially interact with the cell to elicit biological responses. Lipophilic and esterified compounds can freely cross the plasma membrane and produce effects within a cell; other compounds gain entry to the cell only through specific channels or energy-dependent pumps, whereas membrane-impermeable chemicals may stimulate cellular responses by acting on receptors that initiate signaling cascades within the cell. As a result, each chemical may provide a unique stimulus that sets in motion specific signaling pathways. Although no single mechanism explains the increased cell proliferation and/or inhibition of apoptosis observed following conditions that favor increased cellular oxidants, mounting evidence is emerging that links reactive oxygen species with altered expression of growth regulatory genes.

OXIDATIVE STRESS AND GENE EXPRESSION

Although increases in reactive oxygen species production may lead to the induction of apoptosis or necrosis, low levels of oxidants, through interaction and modification of DNA, may alter gene expression. Researchers examining the effects of reactive oxygen species on cell proliferation demonstrated that the induction of cell proliferation occurred only at exposure to low concentrations or transient exposure to reactive oxygen species (106). It has been clearly demonstrated that reactive oxygen species and other free radicals influence the expression of a number of genes and signal transduction pathways.

Many xenobiotics, by increasing cellular levels of oxidants, alter gene expression through a host of signaling pathways including cAMP-mediated cascades,

calcium-calmodulin pathways, and intracellular signal transducers such as nitric oxide, resulting in either cell proliferation or selective cell death (apoptosis or necrosis) (107, 108). Calcium has long been recognized as a signaling factor involved in the regulation of a wide range of processes including cell proliferation, differentiation, and apoptosis (109). Researchers have reported that reactive oxygen species induce the release of calcium from intracellular stores, resulting in the activation of kinases, such as protein kinase C (PKC) (110). PKC can also be activated by H_2O_2 and redox cycling quinones (111). Interestingly, PKC activation is differentially regulated by cellular oxidants: oxidation at the NH_2-terminal regulatory domain activates, whereas oxidation at the COOH terminal inactivates PKC (112). Similarly, H_2O_2 leads to the activation of protein kinase B (PKB/Akt), which is associatied with Hsp27 (113). Cellular receptors for growth modulatory molecules are also affected by reactive oxygen species. Epidermal and platelet-derived growth factor receptors can be activated by oxidizing molecules, resulting in activation of downstream signaling systems, and may participate in the carcinogenesis process (114).

The effects of cellular oxidants have also been related to activation of transcription factors. The most significant effects of oxidants on signaling pathways have been observed in the mitogen-activated protein (MAP) kinase/AP-1 and NF-κB pathways (115). The activation of these transcription factors is involved in both cell proliferation and apoptosis. The cellular concentration of reactive oxygen species appears to influence the selective activation of these transcription factors and therefore may help explain the observation that either cell death or cell proliferation may result from exposure to reactive oxygen species.

AP-1 is a collection of dimeric basic region-leucine zipper (bZIP) proteins that belong to the Jun (c-Jun, JunB, JunD), Fos (FosB, Fra-1, Fra-2), Maf, and ATF subfamilies, all of which can bind TPA or cAMP response elements (117). c-Jun, a potent transcriptional regulator, often forms stable heterodimers with Jun proteins, which aid the binding of Jun to DNA (118). AP-1 activity is induced in response to H_2O_2 as well as several cytokines and other physical and chemical stresses. In addition, in vitro transcriptional activity of AP-1 is regulated by the redox state of a specific cysteine located at the interface between the two c-Jun subunits, highlighting the importance of redox status on gene transcription (119). The stressors (H_2O_2 or otherwise) invoke a signal cascade that begins with the activation of MAP kinases (120).

MAP kinases, a family of serine/threonine kinases, regulate processes important in carcinogenesis including proliferation, differentiation, and apoptosis. Three major subfamilies have been identified: extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), and the p38 kinases (121). MAP kinases modulate gene expression through phosphorylation of a wide array of transcription factors. Of the three subfamilies, the ERK pathway has most commonly been associated with the regulation of cell proliferation. Activation of the ERK, JNK, and p38 subfamilies has been observed in response to changes in the cellular redox balance. The balance between ERK and JNK activation is a key determinant for

cell survival as both a decrease in ERK and an increase in JNK is required for the induction of apoptosis (122).

The induction of AP-1 by H_2O_2, cytokines, and other stressors is mediated mainly by JNK and p38 MAP kinase cascades (120). Once activated, JNK proteins translocate to the nucleus and phosphorylate c-Jun and ATF2, enhancing transcriptional activities (123, 124). H_2O_2 can activate MAP kinases and thereby AP-1 in several manners. One involves a MAP kinase kinase, apoptosis signal-regulating kinase (ASK1) (125). It has been suggested that ASK1 activity is inhibited by thioredoxin. Oxidation of thioredoxin by H_2O_2 is thought to disrupt ASK1 inhibition, resulting in ASK1 activation (125). The other mechanism involves oxidant-mediated inhibition of MAP kinase phosphatases, which leads to increased MAP kinase activation. In either mechanism, activation of MAP kinases directly leads to increased AP-1 activity. The relevance of AP-1 activation, and the role of cellular oxidants in the activation in the cancer process, has been evidenced by a number of observations.

A common effect of AP-1 activation is an increased cell proliferation. In particular, several lines of evidence have demonstrated that c-fos and c-jun are positive regulators of cell proliferation (126). One of the genes regulated by AP-1 is cyclin D1. AP-1 binding sites have been identified in the cyclin D1 promoter and AP-1 activates this promoter, resulting in activation of cyclin-dependent kinase, which promotes entry into the cell division cycle (127). c-Jun also stimulates the progression into the cell cycle both by induction of cyclin D1 and suppression of p21waf, a protein that inhibits cell cycle progression (128). JunB, considered a negative regulator of c-jun-induced cell proliferation, represses c-jun-induced cyclin D1 activation by the transcription of $p16^{INK4a}$, a protein that inhibits the G1 to S phase transition (129).

Expression of c-jun and c-fos can be induced by a variety of compounds including nongenotoxic and tumor promoting compounds (carbon tetrachloride, phenobarbital, TPA, TCDD, cadmium, alcohol, ionizing radiation, asbestos), many of which generate reactive oxygen species (130–133). The induction of c-jun and c-fos expression from reactive oxygen species generated by ionizing radiation can be inhibited by NAC (134). In addition to affecting cell proliferation, AP-1 proteins also function as positive and negative regulators of apoptosis. Whether AP-1 induces or inhibits apoptosis is dependent upon the balance between the pro- and antiapoptotic target genes, which may vary from one cell type to another, the developmental stage, the stimulus used to activate AP-1, and the duration of the stimulus (122). Finally, important to neoplastic development, through increased production of growth factors as well as modulation of cell cycle regulators, AP-1 proteins participate in oncogenic transformation through interaction with activated oncogenes such as Ha-ras (135).

NFκB is an inducible and ubiquitously expressed transcription factor for genes involved in cell survival, differentiation, inflammation, and growth (136, 137). Active NFκB complexes are dimers of proteins from the Rel family of proteins consisting of p50 (NFκB1), p52 (NFκB2), c-Rel, v-Rel, Rel A (p65), and Rel B

(138). NFκB is maintained in an inactive state in the cytoplasm by binding to inhibitory IκB proteins. Activation of NFκB occurs in response to a wide variety of extracellular stimuli that promote the dissociation of IκB, which unmasks the nuclear localization sequence and thereby allows entry of NFκB into the nucleus and binds κB-regulatory elements (139). Redox status has also been shown to impact on NFκB regulation. Activation of NFκB has been observed upon *S*-thiolation of cys62 sited at the p50 subunit of the NFκB dimer (140). Although experimental evidence for redox regulation of transcription factors has been demonstrated in vitro, in vivo evidence has been suggested but only indirectly demonstrated. Wu et al. (141) found that oxidative stimuli promoted the oxidation of p53 to a mixed disulphide with GSH and that this modification inhibited transcriptional activity and nuclear localization of NFκB.

NFκB activation has been linked to the carcinogenesis process because of its roles in inflammation, differentiation, and cell growth. NF-κB regulates several genes involved in cell transformation, proliferation, and angiogenesis (142). Carcinogens and tumor promoters including UV radiation, phorbol esters, NNK, asbestos, alcohol, and benzo(a)pyrene are among the external stimuli that activate NFκB (138, 143). Through complex pathways that are still being elucidated, NFκB activation is involved in cell survival. The expression of several genes regulated by NFκB (bcl-2, bcl-x_L, TRAF1, TRAF2, SOD, and A20) promotes cell survival at least in part through inhibition of apoptotic pathways. Expression of NFκB has been shown to promote cell proliferation, whereas inhibition of NFκB activation blocks cell proliferation (144). Additionally, tumor cells from blood neoplasms, and colon, breast, pancreas, and squamous cell carcinoma cell lines have all been reported to constitutively express activated NFκB (145).

The mechanism for activation of NFκB by reactive oxygen species is not clear. Reactive oxygen species have been implicated as second messengers involved in activation of NFκB via tumor necrosis factor (TNF) and interleukin-1 (146). Suppression of TNF and interleukin-1 were shown to downregulate the expression of active NFκB and inhibit proliferation of lymphoma and myelogenous leukemia cells (147). Of note is the fact that protein kinases are also involved in cell response mediated by the TNF superfamily. In fact, the binding of TNF to its receptor is associated with H_2O_2 generation and protein-protein disulphide bond formation (148, 149). Oxidative changes may amplify the TNF receptor-mediated signal, and can function either to activate protein kinases [e.g., stress-activated protein kinase (SAPK), extracellular signal regulated kinase (ERK), and p38] or inhibit transcription factors, such as AP-1 and NFκB (150). Therefore, the decision to commit to cell death or cell survival will in part depend on the strength and duration of oxidant exposure and on the cell type involved.

The importance of reactive oxygen species on NFκB activation is further supported by studies demonstrating that activation of NFκB by nearly all stimuli can be blocked by antioxidants, including L-cysteine, NAC, thiols, green tea polyphenols, and vitamin E (151, 152). That NFκB activation appears to be selectively mediated by peroxides as activation was observed only following exposure to H_2O_2 or

butylperoxide, and not superoxide or hydroxyl radicals (153). Likewise, NFκB activity was increased in cells that overexpressed superoxide dismutase and decreased in cells overexpressing catalase (154). NFκB activation signals the transcription of genes such as manganese containing SOD and γ-GCS. Collectively these findings support the linkage of NFκB activation by reactive oxygen species with the carcinogenesis process.

Activation of transcription factors is clearly stimulated by signal transduction pathways that are activated by H_2O_2 and other cellular oxidants. Through the ability to stimulate cell proliferation and either positive or negative regulation of apoptosis, transcription factors can mediate many of the documented effects of both physiological and pathological exposure to H_2O_2, or chemicals that induce reactive oxygen species and/or other conditions that favor increased cellular oxidants. Through regulation of gene transcription factors, and disruption of signal transduction pathways, reactive oxygen species are intimately involved in the maintenance of concerted networks of gene expression that may interrelate with neoplastic development.

DNA METHYLATION AND CARCINOGENESIS

Methylation status of cellular DNA is considered an epigenetic mechanism that influences gene expression (155). Altered methylation does not involve a change or miscoding of DNA base-coding sequence, but rather leads to aberrant gene expression, in part, by affecting the ability of methylated DNA-binding proteins to interact with *cis* elements (156). PostDNA synthetic methylation of the 5 position on cytosine [5-methylcytosine (5mC)] is a naturally occurring modification to DNA in higher eukaryotes. Under normal conditions, DNA is methylated symmetrically on both strands. Immediately following DNA replication, the newly synthesized double-stranded DNA contains hemimethylated sites that signal for DNA maintenance methylases to transfer methyl groups from S-adenosylmethionine to cytosine residues on the new DNA strand (157). If a cell is signaled to undergo DNA synthesis prior to maintenance methylation, then double-stranded DNA with hypomethylated regions will be propagated in subsequent cell division cycles, giving rise to potentially heritable genetic changes. 5mC in DNA is known to affect gene expression and alteration of cellular processes such as development and differentiation, and appears to be an important mechanism in carcinogenesis (158–160).

During the carcinogenesis process, DNA methylation may be such that both hypomethylation and hypermethylation occur (158, 159). The degree of methylation within a gene inversely correlates with the expression of that gene. Hypermethylation of genes may inhibit transcription of tumor suppressor genes (161) and is associated with decreased gene expression or gene silencing. Important to the cancer process, tumor suppressor genes are known to be hypermethylated and subsequently inactivated (158–160). Progressive increases in methylation of CpG islands have been observed in bladder cancer and specific tumor suppressor genes

have been reported to be methylated in tumors, e.g., the retinoblastoma gene, $p16^{ink4a}$, and $p14^{ARF}$ (162–165). Inactivation of $p16^{ink4a}$ by hypermethylation of the promoter region appears to be an early event in lung cancer (166). Furthermore, in nickel carcinogenesis, hypermethylation of $p16^{ink4a}$ is apparently induced by reactive oxygen species and activation of MAP kinase pathways (167).

Regional hypermethylation may impart molecular changes associated with genetic instability and may participate in the progression of neoplasia. Conversely, hypomethylation is considered an early and frequent event in the carcinogenesis process (168). A hypomethylated gene is considered to possess an increased potential for expression as compared to a hypermethylated gene (169). In addition, hypomethylation has been associated with increased mutation rates. Most metastatic neoplasms in humans have significantly lower 5 MeC than normal tissue (170). Oncogenes can become hypomethylated and their expression amplified (158, 166).

Dietary constituents containing choline and methionine provide the methyl groups used in methylation reactions. Exposure of rats to a choline/methionine-deficient diet results in hepatocellular proliferation and neoplasia (171, 172). The induction of cell proliferation by a methyl-deficient diet appears to function through decreased hepatic levels of S-adenosyl-methionine and, thus, promotes hypomethylation and subsequent expression of oncogenes. Prolonged administration of a diet deficient in choline or methyl donor groups resulted in hypomethylation of c-myc, c-fos, and c-H-ras protooncogenes and was associated with the induction of hepatocarcinogenesis in rodents (172, 173). Also consistent with the role of methylation of DNA in the promotion stage of the carcinogenesis process, the induction of hepatocarcinogenesis by methyl-deficient diets was shown to be reversible by the administration of S-adenosyl-methionine (174, 175).

Among the agents and situations that can alter methylation status, reactive oxygen species can modify DNA methylation patterns. In particular, oxidative DNA damage (176) can result in decreased DNA methylation. Several chemical carcinogens modify DNA methylation, methyltransferase activity, and chromosomal structure. Of particular importance, the formation of oxidative DNA lesions has been linked to changes in DNA methylation profiles and the carcinogenesis process. Oxidative DNA damage can interfere with the ability of methyltransferases to interact with DNA, thus resulting in a generalized hypomethylation of cytosine residues at CpG sites. The formation of OH8dG in DNA by reaction of the hydroxyl radical or singlet oxygen with DNA can lead to hypomethylation of DNA since the presence of OH8dG in CpCpGpGp sequences inhibits the methylation of adjacent C residues. Additionally, OH8dG formation can interfere with the normal function of DNA methyltransferase and alter DNA methylation status (177). Thus, oxidative DNA damage may be an important contributor to the carcinogenesis process brought about by the loss of DNA methylation, allowing the expression of normally quiescent genes. Also, the abnormal methylation pattern observed in cells transformed by chemical oxidants may contribute to an overall aberrant gene expression and promote the tumor process.

The roles of oxidative DNA damage, mutation, and altered gene expression induced by cellular oxidants and/or altered methylation status have been discussed in relation to the carcinogenesis process. Although specific examples exist for each of these pathways, it should be realized that these mechanisms are not mutually exclusive.

OXIDATIVE STRESS AND GAP JUNCTIONAL MODIFICATION

In multicellular organisms, the intercellular exchange of cellular factors from one cell to a neighboring cell is mediated through either extracellular signally molecules (i.e., hormones) or between adjacent cells through gap junctional intercellular communication (178). Gap junctions are comprised of plaques of transmembrane channels composed of connexin hexamers forming a connexin hemichannels. The hemichannels of one cell connect with hemichannels on neighboring cells to form a transmembrane conduit between the two adjacent cells, allowing for the exchange of ions and low-molecular-weight water-soluble materials (less than 1000 Das) between the cells. Growth regulatory and signal transducing substances, including molecules that are involved in the regulation of the cell cycle, cell growth, and cell death, are able to pass through the gap junction. Therefore, the gap junction allows maintenance of a steady level of low-molecular-weight messenger molecules among cell populations.

Gap junctional intercellular communication can be modulated during the cancer process (179). Cell lines established from tumors of both animal and human tissues exhibit decreased gap junctional intercellular communication. During multistage rat hepatocarcinogenesis, gap junctional intercellular communication progressively decreases. Additionally, liver carcinomas have also displayed reduced ability to communicate with neighboring cells (180). Control of growth regulation in tumorigenic cells lacking gap junctional intercellular communication is restored by transfection with connexin genes. Blockage of cell-to-cell communication has particularly been associated with the tumor promotion stage of chemical carcinogenesis and has been suggested as a mechanism of action for the tumor promotion process (181, 182). Many tumor-promoting compounds demonstrate a tissue- and species-specific inhibition of gap junctional intercellular communication in vivo and in vitro following exposure (183). A correlation between the ability of a compound to block cell-to-cell communication in cultured cells and its ability to induce rodent tumors through nongenotoxic mechanisms has also been demonstrated (183). Additionally, the inhibition of gap junctional intercellular communication observed following treatment with tumor promoting compounds correlates with species and strain sensitivity of the chemical (181, 184).

The induction of oxidative stress modulates cell-to-cell communication. Hydrogen peroxide (H_2O_2), an established tumor promoter, inhibits intercellular communication that is reversible and involves a glutathione-dependent signal transduction

pathway (185, 186). In addition to the direct action of active oxygen species on gap junctional intercellular communication, a number of chemicals that produce reactive oxygen species also inhibit intercellular communication in a variety of cells in culture. The phorbol ester and tumor promoter 12-o-tetradecanoylphorbol-13-acetate (TPA) has also been shown to produce H_2O_2 in murine epidermal keratinocytes and is known to block intercellular communication. Similarly, DDT and paraquat, nongenotoxic liver carcinogens, selectively block intercellular communication in isolated mouse hepatocytes (181, 184). Additionally, carbon tetrachloride inhibition of cell-to-cell communication in rat hepatocytes occurs through an oxidative stress–mediated process. Further support for the involvement of reactive oxygen species in the inhibition of gap junctional intercellular communication by liver tumor promoters comes from studies examining antitumor-promoting compounds and/or cancer chemopreventive agents that function as antioxidants. Vitamin E and the green tea catechin, EGCG, abolish the inhibition of intercellular communication in mouse hepatocytes following treatment with phenobarbital and DDT (184). Thus, one function of antioxidants may be to maintain expression of gap junctions in cells. Additionally, alteration in cellular oxidative stress status appears to affect the assembly of gap junctions in neoplastic nodules (187).

The mechanism for the involvement of gap junctional intercellular communication in the carcinogenesis process may relate to the fact that the gap junction mediates the passage of both positive and negative growth regulatory molecules between neighboring cells. Blockage of gap junctional intercellular communication between normal and preneoplastic cells creates an environment in which preneoplastic cells are isolated from growth controlling factors of normal surrounding cells (182). Inhibition of gap junctional intercellular communication would therefore be expected to be involved in decreased regulation of homeostatic growth control allowing the preneoplastic cell to escape the growth control of normal surrounding cells, resulting in clonal expansion.

SUMMARY AND CONCLUSIONS

A linkage between an increase in cellular reactive oxygen radicals and the pathogenesis of several chronic diseases including cancer has been established. Cellular oxidants (reactive oxygen and nitrogen species) can be generated from endogenous (normal physiological processes) as well as exogenous sources (xeonbiotic interaction). When the antioxidant control mechanisms are exhausted or overrun, the cellular redox potential shifts toward an oxidative stress, in turn, increasing the potential for damage to cellular nucleic acids, lipid, or protein. Unrepaired damage to DNA may result in mutation, provided cell replication ensues prior to repair of modified bases. Although importance has been established for the role of oxidative nuclear DNA damage in neoplasia, formation of mitochondrial DNA damage, mutation, and alteration of the mitochondrial genomic function also appear to contribute to the process of carcinogenesis.

Besides direct modification of nuclear and/or mitochondrial DNA, interference with oxidative DNA repair mechanisms (in both nuclear and mitochondrial compartments) contributes to an increase in mutation frequency and persistent oxidative DNA damage. Aside from a role of oxidants in the induction of mutation, it is apparent that reactive oxygen species and cellular redox status mediate cell signaling pathways that are involved in cell growth regulatory pathways and, thus, the carcinogenesis process. The role of reactive oxygen species in cell growth regulation is complex, being cell specific and dependent upon the form of the oxidant as well as the concentration of the particular reactive oxygen species. The modification of gene expression by reactive oxygen species has direct effects on cell proliferation and apoptosis through the activation of transcription factors including mitogen-activated protein (MAP), kinase/AP-1, and NF-κB pathways. Oxidant-mediated AP-1 activation results in enhanced expression of cyclin D1 and cyclin-dependent kinase, which in turn promotes entry into mitosis and cell division. Likewise, reactive oxygen species function as second messengers involved in activation of NFκB by tumor necrosis factor and cytokines. Gene expression is also controlled by the methylation status of genomic DNA. Recent evidence shows that reactive oxygen species as well as oxidative DNA damage modulate DNA methylation patterns, and may contribute to the multistage carcinogenesis process. DNA damage, mutation, and altered gene expression are all required participants in the process of carcinogenesis. Although these events may be derived by different mechanisms, a commonality is the involvement of cellular oxidants in neoplastic development.

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