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INFLAMMATION AND LUNG CANCER: ROLES OF REACTIVE OXYGEN/NITROGEN SPECIES

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The lung is a highly specialized organ that facilitates uptake of oxygen and release of carbon dioxide. Due to its unique structure providing enormous surface area to outside ambient air, it is vulnerable to numerous pathogens, pollutants, oxidants, gases, and toxicants that are inhaled continuously from air, which makes the lung susceptible to varying degrees of oxidative injury. To combat these unrelenting physical, chemical, and biological insults, the respiratory epithelium is covered with a thin layer of lining fluid containing several antioxidants and surfactants. Inhaled toxic agents stimulate the generation of reactive oxygen/nitrogen species (ROS/RNS), which in turn provoke inflammatory responses resulting in the release of proinflammatory cytokines and chemokines. These subsequently stimulate the influx of polymorphonuclear leukocytes (PMNs) and monocytes into the lung so as to combat the invading pathogens or toxic agents. In addition to the beneficial effects, persistent inhalation of the invading pathogens or toxic agents may result in overwhelming production of ROS/RNS, producing chronic inflammation and lung injury. During inflammation, enhanced ROS/RNS production may induce recurring DNA damage, inhibition of apoptosis, and activation of proto-oncogenes by initiating signal transduction pathways. Therefore, it is conceivable that chronic inflammation-induced production of ROS/RNS in the lung may predispose individuals to lung cancer. This review describes the complex relationship between lung inflammation and carcinogenesis, and highlights the role of ROS/RNS in cancer development.

Human lung is highly vascularized so as to facilitate gaseous exchange by its well-coordinated interaction with the central nervous system, the diaphragm/chest wall musculature and the circulatory system. The adult human lung provides an enormous surface area of approximately 140 m^2 to air. This makes the lung vulnerable to a wide range of toxicants and infectious agents with the potential to induce oxidative damage. An average adult inhales about 10,000 liters of air per day, polluted with cigarette smoke, automobile exhaust, diesel soot, ozone (O_3), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and varying degrees of other pollutants (D'Amato et al., 2002; Schwela, 2000). Inhalation of such toxic air pollutants and microorganisms results in lung injury and generation of reactive oxygen/nitrogen species (ROS/RNS), leading to cascades of signaling events that trigger production of proinflammatory cytokines and chemokines (Emmendoerffer et al., 2000). Inflammation is the primary reaction of a tissue to eliminate pathogenic insult and injured tissue components in order to restore normal physiological functions or replace the irreparable tissue with scar tissue. Phagocytic alveolar macrophages (AMs), polymorphonuclear leukocytes (PMNs), and eosinophils are integral to the innate defense mechanisms of the lung (DosReis & Borges, 2003). These cells play a pivotal role in eliminating the pathogen or biological insult through the generation of various reactive species including superoxide ($\bullet O_2^-$), nitric oxide ($\bullet NO$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet OH$), peroxynitrite ($ONOO^-$) and hydrochlorous acid ($HOCl\bullet$) (Emmendoerffer et al., 2000). These reactive species help in killing the pathogen, and inflammation often subsides after the assaulting agent is removed or following completion of the repair process. However, repeated tissue damage and regeneration produce increased ROS/RNS from inflammatory cells, which then interact with DNA in proliferating epithelium, resulting in permanent

The statements and conclusions made in this report are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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genomic alterations such as point mutations, deletions, or rearrangements (Coussens & Werb, 2002). Although cells respond to DNA damage by activating p53-controlled genes associated with cell cycle and DNA repair, when the rate of ROS/RNS-mediated DNA damage is extensive, it leads to chronic inflammation. Chronic inflammation provides a microenvironment rich in (1) inflammatory cells, (2) ROS/RNS, (3) recurring DNA damage, (4) cell-proliferating growth factors and (5) other growth-supporting stimuli that increases the frequency of mutations. This further facilitates progressive transformation of cells to the malignant form (Cook et al., 2004), increasing the risk of developing lung cancer (Ardies, 2003). In pulmonary pathologies such as chronic obstructive pulmonary disease (COPD), fibrosis, and particulate and chemical-induced lung carcinogenesis, inflammation is considered as a major precursor or the "hallmark" for cancer development. In this review, the key events triggered by lung inflammation-induced by ROS/RNS generation and their purported role in the genesis of lung cancer are discussed.

REACTIVE OXYGEN/NITROGEN SPECIES

ROS/RNS are the key players in lung inflammation and cancer as their increased production leads to genetic mutations, predisposing individuals to cancer. Cells are constantly exposed to ROS/RNS generated from endogenous and some exogenous sources. Aerobic metabolism is the major source of ROS/RNS, which are produced in almost all tissues and participate in normal cell functions. ROS/RNS act as intracellular signaling molecules in many biological processes (Chatterjee & Fisher, 2004; Hancock et al., 2001). Low concentrations of ROS/RNS were found to (1) activate transcription factors such as nuclear factor kappa B (NF-κB), activator protein-1 (AP-1), (2) induce apoptosis or necrosis, and (3) alter mitogenic signals that affect cell growth and cell signaling cascades (Sauer et al., 2001; Taniyama & Griendling, 2003). On the other hand, increased concentrations of ROS/RNS are associated with initiation and aggravation of various pathologic conditions (Chatterjee & Fisher, 2004). The mutagenic potential of ROS depends on the reactivity and diffusability of these species (Aust & Eveleigh, 1999). It was estimated that approximately 2% of inhaled oxygen was converted to reactive oxygen by various biological reactions with the potential to induce protein and DNA damage (Campa et al., 2004).

Among the various types of reactive species produced during normal and inflammatory responses, $\bullet O_2^-$ is considered as the primary ROS. During phagocytosis and ingestion of pathogens or foreign materials, the cell membrane-bound multi-subunit enzyme complex of NADPH oxidase is activated, resulting in one-electron reduction of molecular oxygen to $\bullet O_2^-$. $\bullet O_2^-$ is then dismutated by a family of enzymes known as superoxide dismutase (SOD) to produce H_2O_2 (Hancock et al., 2001). The physiological danger comes from the ability of H_2O_2 to generate highly reactive •OH radicals in the presence of redox-active transition metals such as iron (Fe^{2+}) or copper (Cu^+) (Shacter & Weitzman, 2002). •OH radicals react spontaneously, causing DNA strand breaks and base modifications (Shacter & Weitzman, 2002). HOCl•, formed from H_2O_2 and chloride in a reaction catalyzed by myeloperoxidase activated by neutrophils, is also a potent oxidizing agent that produces DNA damage. Furthermore, RNS such as NO• can induce guanine nitration, producing G:C to T:A transversions, and thus are involved in inflammation-induced carcinogenesis (Ohshima et al., 2006; Terasaki et al., 2006). The products of nitric oxide synthesis induce mutations through *N*-nitrosation of secondary amines. These *N*-nitrosamines are markedly mutagenic and hence may play a critical role in carcinogenesis induced during chronic inflammation (Szabo & Ohshima, 1997). In addition to its key role in producing •OH radicals, $\bullet O_2^-$ rapidly reacts with NO• to produce peroxynitrite ($ONOO^-$) (Grisham et al., 2000). Akin to •OH radicals, $ONOO^-$ produces DNA base modifications, DNA strand breaks, and mutations through oxidative mechanisms (Szabo & Ohshima, 1997). Figure 1 is a schematic representation of the major cellular reactions involved in the production of different reactive species. In addition to the phagocytic source, nonphagocytic NADPH oxidases are considered important sources of ROS, particularly $\bullet O_2^-$, in nonphagocytic tissues. NADPH oxidases are comprised of membrane-bound $gp91^{phox}$ and $p22^{phox}$ and cytosolic $p47^{phox}$, $p67^{phox}$, $p40^{phox}$, and Rac^1 or Rac^2 (Lassegue & Griendling, 2002). NADPH oxidases are structurally related to neutrophil oxidase but exhibit much lower activity and different substrate

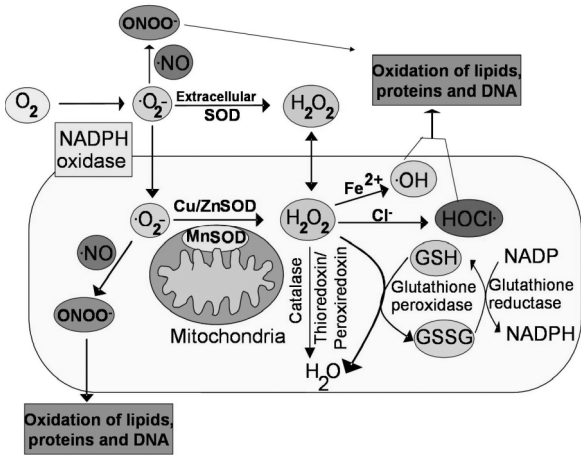

FIGURE 1. Schematic representation of the major pathways involved in the intracellular production of reactive oxygen/nitrogen species.

specificity (Li & Shah, 2003). These enzymes are dormant and may function as second messenger in redox signaling; however, when stimulated appropriately they produce higher levels of ROS that may contribute to oxidative stress. Evidence suggest that nonphagocytic NADPH oxidases are major source of ROS generation in hypertension, inflammation, and atherosclerosis (Griendling & Ushio-Fukai, 2000; Zalba et al., 2001).

ROS/RNS produced during different cellular reactions may be either beneficial or harmful to the cells, thus, acting as a "double-edged sword" in cellular reactions. ROS and RNS induce damage to macromolecules such as DNA, lipids, and proteins through their ability to induce biochemical alterations (Taniyama & Griendling, 2003; Wu et al., 2004). However, these species are also important in many physiological functions, and it is well recognized that the balance between oxygen utilization, ROS/RNS formation, and antioxidant activity is essential for normal functions of the body.

LUNG INFLAMMATION

Physical, chemical, or biological injury to the lung leads to inflammatory response, which is initiated as a protective mechanism to get rid of the organism or agents causing it. Inflammation triggers a series of cellular events that try to heal the damage. Inflammation is classified as acute or chronic inflammation, depending on a variety of factors including clinical symptoms and the nature of injury. Acute inflammation is the immediate response, usually of short duration, and results in the release of PMNs so as to eliminate the pathogenic or cytotoxic insult. On the other hand, chronic inflammation is characterized by persistent inflammation, tissue injury, and tissue repair, occurring simultaneously. The inefficiency of the biological mechanisms in resolving the inflammatory response results in chronic conditions. Several pathophysiologic states and inhaled foreign particulates are known to produce sustained stimulation of phagocytic cells, resulting in the upregulation of proinflammatory cytokines and chemokines, provoking chronic inflammation in the lung (Lang et al., 2002).

Lung consists of several different cell types such as type I and type II pneumocytes, Clara cells, mast cells, and ciliated cells (Emmendoerffer et al., 2000). In addition, free migratory AMs, PMNs, eosinophils, and lymphocytes represent normal immune defenses of the lung. Different cell types are recruited to respond to different infections and toxicants, depending on the inducing agent or the type of inflammation. For instance, Type II pneumocytes and bronchial epithelial cells generally proliferate to preneoplastic lesions in response to carcinogens leading to the development of lung cancer (Emmendoerffer et al., 2000). Additionally, ROS produced by nonphagocytic oxidases also affect cell recruitment to the sites of inflammation by regulating adhesion molecule expression on

inflammatory cells (Fraticelli et al., 1996; Niu et al., 1994). In acute inflammatory response, PMNs and in some cases eosinophils are the primary recruited effectors. Monocytes, which differentiate into macrophages in tissues, are the next to migrate to the site of tissue injury, guided by chemotactic factors (Coussens & Werb, 2002). Once activated, macrophages produce reactive species, growth factors, and cytokines, which profoundly affect endothelial, epithelial, and mesenchymal cells in the focal area, leading to disorganization of the lung microenvironment. Mast cells also play an important role in acute inflammation owing to their ability to release inflammatory mediators such as histamines and cytokines (Cook et al., 2004). The failure to regulate acute inflammation focally results in loss of control of these responses with the development of chronic inflammation, and in some cases may lead to malignant transformation (Marshall, 2001). Therefore, even though inflammation helps in regaining the normal physiological function of the tissue, it may also lead to harmful effects such as neoplastic development. Acute inflammation is often transient and thus is not implicated in the genesis of cancer. The key concept is that normal inflammation is usually self-limiting; however, dysregulation of any of the converging factors leads to abnormalities and neoplastic progression (Coussens & Werb, 2002).

LUNG CANCER

Lung cancer is the leading cause of cancer mortality worldwide, with over 1,000,000 deaths annually (Jemal et al., 2004). In the United States, 160,440 deaths were attributed to cancer of the lung and bronchus in 2003 (Cancer Facts and Figures, 2004). In 2005, 172,570 new cases were diagnosed with lung cancer and 163,510 people died of this disease (Breath, 2006). Lung cancer is rare among young adults, with the average age of occurrence and diagnosis being over 60 yr. There are two major classes of lung cancer: (1) primary lung cancer and (2) secondary lung cancer. Primary lung cancer originates in the lung itself and is further classified into two subtypes: (a) small-cell lung cancer (SCLC) and (b) non-small-cell lung cancer (NSCLC), depending on the morphology of the malignant cells. Secondary lung cancer is initiated in other organs such as breast or colon and then spreads to the lungs.

Current research indicates that long-term exposure to inhaled carcinogens has the greatest impact on risk of lung cancer. Tobacco smoking is considered to be the leading cause of lung cancer, with approximately 85% of deaths directly linked to smoking. Mainly, polyaromatic hydrocarbons (PAHs) and nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are the major components of tobacco that are associated with the etiology of lung cancer (Goud & Kaplan, 1999; Hoffmann & Hoffmann, 1997; Wogan et al., 2004). In recent years, smoking marijuana was also considered to be a risk factor for lung cancer as it can induce lung injury. Although marijuana smoking is not common, it is still deleterious because the way it is smoked leads to greater deposition of smoke carcinogens and particles in the lung as compared to cigarette smoking (Tashkin, 2005). Primary lung cancer incidence linked to tobacco was first suggested by Rottman in German tobacco workers in 1898 (Rottman, 1898). Later, in 1912, Adler, a pathologist in United Kingdom, reported malignant growths of tumors in bronchi and lungs of smokers (Adler, 1912). However, it was conclusively established only in 1939 by Muller, in a case-control study, as a specific risk factor in cigarette smokers (Muller, 1939). Later, various important studies established a dose-response relationship of tobacco smoking and lung cancer (Doll and Hill, 1950; Wynder & Graham, 1950). Besides active smoking, passive smoking (secondhand smoke) is also considered to be a potent risk for lung cancer development. Individuals exposed to particulate and toxic chemicals, such as asbestos, coal, radioactive materials, arsenic, vinyl chloride, mustard gas, auto fumes, diesel soot, and other carcinogens, also have a higher than average risk of contracting lung cancer (Shi et al., 1998). In addition to the exclusive environmental causes of lung cancer, it is probable that there is substantial individual variation in the susceptibility to respiratory carcinogens (Alberg & Samet, 2003). It was reported that people with a family history of lung cancer appear to have a higher risk of contracting the disease as compared to others (Economou et al., 1994; Nitadori et al., 2006; Tokuhata & Lilienfeld, 1963). Given the multistep etiology of lung cancer, synergistic interactions among risk factors may also have substantial consequences for lung cancer risk (Alberg & Samet, 2003).

Inflammation-induced scar tissue often produced in the lung in diseases such as berylliosis, asbestosis, and silicosis may also predispose to and increase the risk of developing lung cancer. Overall, the pathogenesis of lung cancer involves multiple molecular abnormalities accrued over a long period of time. Although a large number of genetic pathways associated with lung cancer are being discovered, the basic molecular mechanisms involved in lung cancer are still not clear.

INFLAMMATION AND LUNG CANCER

The functional role of chronic inflammation in cancer is not novel. In 1863, Rudolf Virchow hypothesized that some irritants associated with tissue injury and resulting cellular inflammation may play a role in cell proliferation and neoplastic development. Based on his observations that normal cellular responses might lead to cancer, he postulated that cancer may develop at sites of chronic inflammation (Balkwill & Mantovani, 2001). In the past few years, extensive research in this field clearly demonstrated that cell proliferation alone does not produce cancer. Unlimited proliferation potential of cells is achieved in an environment that is rich in inflammatory cells, producing abundant ROS/RNS promoting unremitting DNA damage, inactivation of apoptosis, upregulation of growth factors, cytokines, and activation of growth supporting genes (Cook et al., 2004). Recent evidence suggests that infections in tissues with chronic inflammation contribute to about one-third of all the known cancers (Ames et al., 1995). In recent years, increased understanding of the basic mechanisms involved in inflammation and its effects in the physiological system supported Virchow's hypothesis, establishing an important relationship between cancer and inflammation.

The potential contribution of chronic inflammation to human lung carcinogenesis has appeared in several other reviews (Balkwill & Mantovani, 2001; Ballaz & Mulshine, 2003; Coussens & Werb, 2002). Lung tumors develop through a complex process involving various stages such as initiation, promotion, and progression (Hanahan & Weinberg, 2000). Genotoxic carcinogens induce the initiation stage by directly damaging the DNA. In the lung, depending on the type of inflammation, there may be direct genotoxic effects such as DNA damage, mutation, or an indirect effect induced by activated enzymes such as cytochrome P-450 oxidase or flavin monooxidases that produce ROS in the cells, resulting in protein and DNA damage (Petruska et al., 1992). Failure in repairing DNA damage results in point mutations and K-ras mutation (12 G-T transversions). K-ras mutations are an early event in the development of adenocarcinoma of the lung and are present in 30% or more of cases (Lacal et al., 1986; Westra et al., 1993). These mutations produce oncogenes with dominant gain of function and tumor suppressor genes with recessive loss of function. Tumor suppressor genes such as p53 and p16 are other examples of genes mutated in lung cancer (Belinsky, 1998; Bennett et al., 1993). Besides mutations, chromosomal abnormalities, DNA adduct formation, methylation, and acetylation are also characteristic of lung cancer. DNA adduct levels are always higher in cigarette smokers compared to nonsmokers (Wiencke, 2002). Methylated DNA loci were reported both in tumor and sputum of lung cancer cases (Palmisano et al., 2000). It was also suggested that DNA methylation and histone deacetylation may together produce silencing of hypermethylated genes in tumors (Suh et al., 2002).

The second stage in cancer development is promotion, which involves clonal expansion of the initiated cells. These initiated cells may undergo promotion under persistent oxidative stress conditions, forming focal lesions from which invasive cancers may originate (Klaunig et al., 1998). Progression is the final stage, involving the formation of completely malignant cells from an early neoplastic clone via both genetic and epigenetic mechanisms (Shacter & Weitzman, 2002). Malignant cells undergo autonomous uncontrolled proliferation with the aid of suitable promoting factors such as epidermal growth factor receptor (EGFR). EGFR, a transmembrane receptor with intrinsic tyrosine kinase activity, triggers many transcription factors and is activated in lung tumors. Various alteration including mutations, gene amplification, overexpression of receptor ligands, and loss or gain of regulatory mechanisms are involved in enhanced proliferation of abnormal cells. In squamous-cell carcinomas of the lung, EGFR is overexpressed by up to 80%. Recently, gefitinib (Iressa), an EGFR tyrosine kinase inhibitor, showed significant variability and mixed results due to the mutations in the kinase domain of the EGF in different populations (Paez et al., 2004; Fukuoka et al., 2003; Janne et al., 2004).

An important aspect of cancer initiation and progression is genome instability. It was reported that the lack of mismatch at the nucleotide level may lead to microsatellite instability in some forms of lung cancers (Massion & Carbone, 2003). In addition to these endogenous sources of inflammation-induced oxidative stress, exogenous sources such as hyperoxia, radiation, exposure to particulates, and chemical carcinogens are also critical in lung carcinogenesis (Shi et al., 1998).

In a mouse model, the manipulation of macrophage population and its contribution to tumor growth was recently reported to show how inflammation enhances lung cancer development (Malkinson, 2005). In lung cancers associated with nondestructive agents such as asbestos, and silica, chronic inflammation in the lung is persistent because of the inability of the immune system to remove these substances. Many of these agents are reported to modulate and activate various transcription factors, producing changes in cell proliferation, differentiation, apoptosis, and inflammation (Janssen et al., 1997). Such inflammatory responses increase the incidence of epithelial cancers, including mesothelioma and lung carcinoma (Steenland & Stayner, 1997). Cigarette smoke is a complex proneoplastic agent that may act, in part, by inducing a chronic inflammatory condition by delivering an array of genotoxic carcinogens such as nitrosamines, peroxides and many potent oxidants into the lungs (Hoffmann & Hoffmann, 1997). In support of the inflammation hypothesis, Okada (2002) reviewed laboratory studies presenting evidence that inflammation-derived free radicals are potent endogenous mediators for cancer development. Inflammatory cells influence the whole organ in tumor development, regulating the growth, migration, and differentiation of all cell types, including neoplastic cells, fibroblasts, and endothelial cells.

ROS/RNS IN LUNG INFLAMMATION

ROS/RNS regulate several kinases, transcription factors, and apoptosis-regulatory proteins (Schenk et al., 1994; Turpaev, 2002), as well as acting as second messengers in intracellular signal transduction pathways (Gilroy et al., 2003; Los et al., 1995). ROS were considered as the key players in inflammation before RNS were discovered. Over the last few years, it was reported that various cytotoxic and genotoxic insults upregulate the expression and synthesis of inducible nitric oxide synthases (iNOS) (Rao, 2000), further complicating the pathogenesis of inflammation. However, in inflammation it is not known whether ROS and RNS interact with each other in the production of new, highly damaging reactive species. With the current comprehensive understanding about ROS/RNS and their roles in inflammation, it may be stated that the effects of ROS/RNS are beneficial or harmful depending on the cell type, reactive species involved, and physiological conditions (Poli, 2002).

Inflammation may be initiated by an infection or cellular damage, which then stimulates the synthesis and activation by macrophages of interleukin (IL)-6, nuclear factor (NF)-κB, and tumor necrosis factor (TNF)-α, which in turn induce molecules such as p-selectin that recruit more leukocytes to the infected area, which produce ROS/RNS (Johar et al., 2004). ROS such as $\bullet O_2^-$, H_2O_2, •OH, and HOCl• kill the invading organism by halogenation or by protein and lipid peroxidation. On the other hand, increased and continuous production of ROS may lead to tissue damage and inflammation. It is known that the local production of ROS/RNS may lead to the accumulation of p53, and the central involvement of p53 in initiating apoptosis is well established (Morrison et al., 2003). The inhibition of p53-dependent apoptotic mechanisms in inflammatory effector cells may contribute to the transition of the acute inflammatory response into the chronic phase of inflammation. NO• may act as a proinflammatory agent by inducing cyclo-oxygenase (COX) enzyme, or NADPH oxidase activity in myeloid cells, and augmenting the production of IL-1 and TNF (Weinberg, 1998). In contrast, it may also activate cyclic guanosine monophosphate (cGMP), which decreases intracellular calcium levels and lowers platelet and PMN aggregation, thus demonstrating anti-inflammatory activity. This anti-inflammatory effect of NO• was reported to be involved in the inhibition of NF-κB. Therefore, NO• in the form of $ONOO^-$ may mediate inflammation and cell death (Miller & Sandoval, 1999) since NF-κB activity may control the expression of COX-2 (inducible isoform of COX), which is an important mediator of inflammation and tumor promotion (Gupta & Dubois, 2001). The activity of COX-2 enzyme is regulated by the redox status of the cell and

inhibited by antioxidants in human AMs (Hempel et al., 1994; Momin et al., 2003). Based on these observations, it may be postulated that conditions of oxidative stress and antioxidant imbalance impair the capacity of macrophages to adequately contribute to the resolution of the inflammatory processes. A balance between inflammation-mediated oxidative damage to tissues and oxidant-induced protective mechanisms is the most desirable state.

ROS/RNS IN LUNG CANCER

An imbalance between cell division and cell repair of a damaged cell increases the rate at which mutations are induced leading to an elevated risk for developing cancer. All cancer cells acquire permanent functional changes to the DNA that confer a selective growth or survival advantage to the cell. Exposure of cells to activated phagocytes leads to oxidative modification of bases, as well as single-strand breaks. ROS/RNS-mediated DNA adduct formation is a component of endogenous carcinogenic risk. ROS/RNS produced by inflammatory cells also stimulate oncogenes such as jun and fos. Overexpression of jun was reported to be directly associated with lung cancer (Szabo et al., 1996; Volm et al., 1994). Proteins and lipids are also significant targets for oxidative attack, and modification of these molecules may increase the risk of mutagenesis (Roberts & Morrow, 1995; Shacter, 2000). For example, oxidative modification of lipids induces mutagenesis through the formation of genotoxic lipid peroxidation by-products that react with the DNA. Protein oxidation may indirectly promote mutagenesis through oxidative modification of DNA polymerase or inhibition of DNA repair enzymes (Marnett, 2000; Wiseman & Halliwell, 1996). Further processing of damaged DNA leads to proneoplastic mutations, including point mutations, deletions, and chromosomal translocations. Despite the presence of a wide array of mechanisms to repair oxidatively damaged DNA, the repair process is slow and not always complete (Shacter et al., 1988). It was demonstrated that the levels of a hydroxyl-mediated DNA adduct, 8-hydroxydeoxyguanosine (8-OH-dG), are elevated in mice treated with NNK, a tobacco-specific carcinogen (Chung & Xu, 1992). However, 8-OH-dG levels dropped in lung DNA on treatment with (–)-epigallocatechin gallate (EGCG), an antioxidant found in green tea, and the incidence of cancer decreased in NNK-treated animals (Chung & Xu, 1992). Another report suggested that deprivation of vitamin A in cattle resulted in increased incidence of lung cancer (Wolbach & Howe, 1978). This relationship was further well established in animals with vitamin A deficiency and benzo[a]pyrene exposure, resulting in increased lung cancer and a decrease in lung cancer in smokers with dietary supplementation of vitamin A and carotenoids (Dogra et al., 1985; Genta et al., 1974; Peto et al., 1981; Wolbach & Howe, 1978). This was followed by the discovery that out of all retinoic acid receptors (RARs), RARβ was the most frequently lost in lung cancer functioning as a tumor suppressor (Xu et al., 1997). Khuri et al. (2000, 2001) demonstrated that in stage I lung cancer, RARβ expression correlated with increased expression of COX-2 and carcinogenesis. However, in several other studies retinoid was reported to be ineffective in reversing premalignancy in individuals who continued smoking. This discrepancy was clarified by a study that evaluated RARβ promoter methylation in tissue specimens from 342 operable NSCLC patients (Kim et al., 2004). The investigators found that in current smokers, second primary lung cancers (SPLCs) developed more frequently when RARβ was unmethylated, and hypermethylation resulted in more lung cancers in former smokers. Increased oxidative stress in current smokers was implicated in the development of SPLC. Apoptosis induced by ROS may be inhibited by RARβ expression (unmethylated RARβ). Therefore, RARβ functions either as an apoptosis inducer and carcinogenesis inhibitor in individuals who quit smoking or as a carcinogenesis enhancer and apoptosis inhibitor in those who continue smoking (Kim et al., 2004). However, the role of retinoids as chemopreventive agents is still being debated.

PROBABLE MECHANISMS OF ROS/RNS-INDUCED CARCINOGENESIS

Lung cancer induced by exposure to cigarette smoke and occupational carcinogens such as asbestos, arsenic, beryllium, cadmium, chromium, diesel, nickel, radon, silica, and vanadium are some examples showing a direct link between ROS/RNS, inflammation, and carcinogenesis.

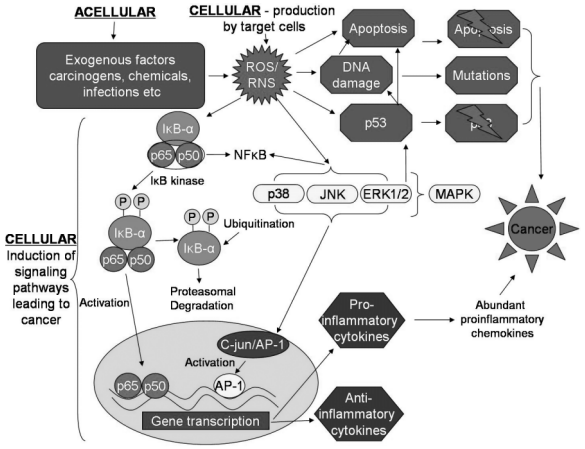

FIGURE 2. Diagrammatic illustration of key molecular events and signaling pathways induced by ROS/RNS in response to various cytotoxic and genotoxic insults. These events either directly or indirectly trigger chronic inflammation leading to carcinogenesis.

In addition, molecular mechanisms implicated in initiating pulmonary inflammatory and carcinogenic responses are also analogous with their ROS/RNS generating potential and all of these agents possess high oxidant producing capacity. Oxidants activate transcription factors such as NF-κB, AP-1, MAPKs, phosphoinositide kinase (PI3K), and activated serine-threonine kinase (AKT) and produce histone acetylation and deacetylation. All these mechanisms are implicated in oxidant-induced carcinogenesis, and some of the important ones that are directly influenced by ROS/RNS and involved in carcinogenesis are briefly described next. Figure 2 is a general schema of some of the key molecular events activated by the enhanced production of ROS/RNS in response to inhaled toxicants or agents, resulting in chronic inflammation and lung cancer.

DNA Damage

Several studies showed that ROS/RNS directly interact with DNA, producing structural alterations including small-scale insertions, DNA base pair deletions, base modifications, chromosomal changes/loss, microsatellite instability, and translocation of segments (Wiseman & Halliwell, 1996). As a result of the normal aerobic metabolism, even without oxidative stress, the oxidants generated are known to produce hundreds of hits per cell per day, inducing more than 100 different oxidative modifications in DNA (Poulsen et al., 1998). In general, •OH radical is the major source of DNA damage that attacks phosphate, deoxyribose, and base sites, resulting in strand breakage. These oxidatively damaged DNA lesions are efficiently excised by a DNA glycosylase (AP lyase). The hydroxylation of guanine in the 8-position is the frequent mutagenic lesion induced by oxidative species. Furthermore, it was established that DNA damage produced by activated phagocytes is a result of attack by •OH radical (Knaapen et al., 1999; Schraufstatter et al., 1988). RNS generated by inflammatory cytokines may induce point mutations and may also damage some DNA repair proteins (Jaiswal et al., 2000). Earlier, it was proposed that cancers may have a mutator phenotype, owing to the disparity between the rarity of spontaneous mutations and the large numbers of mutations reported in various human cancers. Among the thousands of mutations that ensue in a cancer, mutations in oncogenes and tumor suppressor genes are the major ones that confer a malignant phenotype.

Inhibition of Apoptosis

Apoptosis or programmed cell death modulated by p53 is a normal physiological process by which unwanted or damaged cells are eliminated during development and other normal biological processes. It is an ATP-dependent process characterized by membrane blebbing, cell shrinkage, nuclear condensation, and breakdown of DNA, and is important in the genesis of cancer. Disruption of the balance between the rate of cell division and cell death in normal tissues in favor of

excessive growth indicates possible neoplastic development (Shacter & Weitzman, 2002). Various types of cancers use increased survival and decreased death strategies to evade apoptosis and sustain autonomous proliferation. In the case of chronic inflammation-induced cancers, neoplastic development from inflammatory cells may be prevented by inducing apoptosis in inflammatory effector cells (leukocytes) followed by the removal of the apoptotic bodies by phagocytosis (Lauber et al., 2004; Maderna & Godson, 2003). This may exert an anti-inflammatory effect in the chronically inflamed tissue. ROS/RNS are potent intrinsic stimuli for apoptosis that activate and modulate apoptosis when cells are under stress. ROS induce intrinsic apoptosis by mitochondrial damage, release of cytochrome C into the cytoplasm (Reed, 1997), loss of the anti-apoptotic protein Bcl-2 (Kane et al., 1993), degradation of mitochondrial encoded mRNA, rRNA, and DNA (Crawford et al., 1998; Crawford et al., 1997), and reduced transcription of the mitochondrial genome (Kristal et al., 1994). ROS also stimulate proapoptotic signaling molecules, including apoptosis signal-regulating kinase 1 (ASK1), c-Jun N-terminal kinase (JNK), and p38 (Benhar et al., 2001). Dysregulation of apoptosis induced by ROS in various cell types may lead to neoplastic evolution. On the other hand, RNS exhibit both pro- and antiapoptotic roles depending on the type of cells involved, cellular redox state, and the dose of •NO (Heigold et al., 2002). However, the apoptotic pathways and the key molecular targets activated by •NO are not well established. Recent evidence indicates that the proapoptotic activity of •NO involves p38/MAPK, mitochondrial death receptor, and GAPDH cell death pathways (Borutaite & Brown, 2003; Fukuo et al., 1996; Hara & Snyder, 2006; Kuzushima et al., 2006; Li et al., 2004). The mechanisms involved in the antiapoptotic role of •NO include inactivation of caspases, induction of p53 gene expression, and upregulation of anti-apoptotic proteins such as Flip, Bcl-2, and Bcl-X_L (Azad et al., 2006; Chanvorachote et al., 2005; Delikouras et al., 2001; Dimmeler et al., 1997; Kim et al., 1997; Mannick et al., 1994, 1999). Therefore, RNS either produces beneficial effects or exerts deleterious effects.

Activation of Cell Signaling Cascades

ROS/RNS were shown to function as important modulators of signaling mechanisms in several pulmonary diseases including cancer. ROS/RNS-mediated signaling activates pathophysiological events such as cell proliferation, apoptosis, cytokines, and transcription of several genes that are often kept at baseline activity or silent. The signaling cascades triggered by ROS/RNS lead to the activation and phosphorylation of MAPKs, including ERK. This consequently results in activation of transcription factors including NF-κB and AP-1 that may lead to the induction of early response genes such as c-jun and c-fos, which are involved in inflammatory influx, inhibition of apoptosis, cell proliferation, transformation, differentiation, and other changes (Marshall et al., 2004). Oxidative stress induced signaling events that lead to the activation of MAPKs, NF-κB, AP-1, and other key cytoplasmic proteins are presented schematically in Figure 2 (Allen & Tresini, 2000; Ding et al., 2000). The mechanisms associated with oxidative upregulation of these factors are usually induced by the modification of proteins and sulfhydryl groups (Ischiropoulos, 1998). This posttranslational modification of proteins and thiols by ROS/RNS is important in cellular redox signaling. When these alterations are persistent, molecular changes resulting from these pathways elicit preneoplastic progression of cells that eventually results in carcinogenesis.

Activator Protein 1 and Nuclear Factor Kappa B

AP-1 and NF-κB are important transcription factors that are sensitive to ROS/RNS. Oxidants and some inflammatory cytokines such as TNF-α were reported to (1) activate NF-κB and AP-1 and (2) modulate the expression of both proinflammatory and antioxidant genes (Rahman et al., 2002) (Figure 2). This oxidant-mediated gene expression was proposed to be regulated by the degree of acetylation of histones to facilitate DNA binding. In contrast, •NO was implicated in the suppression of NF-κB activation by limiting degradation of IκB (Thomassen & Kavuru, 2001).

AP-1 is composed of oncogene proteins such as c-jun and c-fos and is activated by oxidants such as H_2O_2. Activation plays an important role in the induction of neoplastic transformation and initiation of several genes involved in cell proliferation, differentiation, apoptosis, inflammation, and carcinogenesis (Ding et al., 1999; Ding et al., 2001; Suzuki et al., 1994). It was reported by

Wilhelm et al. (1997) that perturbation of cellular thiol redox status induces stress-activated signal transduction pathways by JNK and p38 kinase that in turn provide a signal for AP-1 activation (Figure 2). This activation led to the induction of genes for cytokines, chemokines, and various proinflammatory mediators that play an important role in the inflammatory response (Devalia & Davies, 1993; Rahman & MacNee, 1998). NF-κB is another vitally important transcription factor that governs the expression of several genes involved in cell development, intercellular communications, apoptosis, and carcinogenesis (Akira & Kishimoto, 1997). In cells, NF-κB is normally bound to the inhibitory protein IκBα in the cytoplasm. ROS/RNS activate NF-κB by rapid phosphorylation, ubiquitination, and subsequent proteasomal degradation of the inhibitory protein (IκBα) This is followed by the translocation of NF-κB to the nucleus, where it activates gene transcription (Figure 2). NF-κB regulates the expression of many genes involved in lung inflammation including iNOS, IL-1β, TNF-α and IL-6, IL-8, E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) (Akira & Kishimoto, 1997; Brennan et al., 1995; Ward, 1996). Persistent production and elevated levels of ROS/RNS result in the activation of NF-κB and AP-1, which through the activation of various proinflammatory cytokines produces chronic inflammation that subsequently culminates in tumor development.

Tumor Suppressor Gene p53

It is well established that p53 is an important tumor suppressor gene involved in cell cycle arrest and many other complex signaling pathways. In case of cell injury (DNA damage), p53 controls surveillance by slowing the progression of cell cycle and initiating apoptosis. p53 induces DNA repair genes such as gad 45 and is thus involved in DNA repair (Enoch & Norbury, 1995). Activation of p53 is also known to be associated with increased production of reactive species and enhanced apoptosis (Polyak et al., 1997). In oxidative stress, p53 is activated leading to DNA damage and apoptosis (Figure 2). In lung cancers, p53 is often mutated and becomes defective in inducing apoptosis. When it is mutated, p53 accumulates in the cytoplasm and functions as an oncogene (Stewart & Pietenpol, 2001). In human lung cancer, p53 is the most frequently altered gene with an overall frequency of 50–55%, most commonly seen in squamous carcinoma and small-cell carcinoma of the lung. Mutations are primarily G to T alterations produced by DNA adduct formation with carcinogens such as polycyclic hydrocarbons frequently found in the lungs of smokers (Bennett et al., 1993; Denissenko et al., 1996).

Cellular Defense Systems

Aerobic organisms possess intrinsic enzymatic and nonenzymatic antioxidant systems to mitigate the damaging effects of ROS/RNS. Enzymatic systems include catalases, peroxidases, thioredoxins (scavenges H_2O_2) and superoxide dismutases (scavenges O_2) [Figure 1] (Scandalios, 1997). These enzymes catalyze decomposition of reactive species into compounds that are harmless to the cellular system. For instance, catalase converts H_2O_2 to H_2O and O_2 before it produces the deleterious •OH radicals. These compounds act directly by quenching free radicals before they damage cellular components. It was shown that the GSH–GSSG antioxidant system protects macrophages from large amounts of NO• produced by iNOS (Coleman, 2001). Nonenzymatic defenses include compounds such as carotenoids, flavonoids, and vitamins E and C. The nonenzymatic antioxidants possess potent antioxidant properties that protect the body against both endogenous and exogenous oxidative stresses (Khan, 2002). The enzymatic systems are intrinsic and are not supported much by our diet, whereas the nonenzymatic antioxidants are primarily derived from diet. The lung has a well-developed biological defense system comprising efficient antioxidant mechanisms against oxidative/nitrosative damage. Several antioxidants are present in the lung epithelial lining fluids. These include reduced glutathione, ascorbic acid, taurine, uric acid, α-tocopherol, ceruloplasmin, antioxidant enzymes, albumin, and proteins. However, overwhelming ROS/RNS production results in oxidative stress. Reports demonstrate that vitamin C protects against pancreatic, lung, stomach, and oral cancer (Khan, 2002). Vitamin E levels are elevated in response to oxidative stress in lung. Intake of vegetables and fruits was also associated with a reduced risk of lung cancer (Cooper et al., 1999). Marchand

et al. (2000), in their case-control study on 582 lung cancer patients, demonstrated an inverse relationship between lung cancer and flavonoids such as quercetin (onions and apples) and naringin (white grapefruit). However, the correlation between cancer prevention and antioxidants is not well established.

CONCLUSIONS

Balanced production of ROS/RNS is critical for normal aerobic metabolism and functioning of several key signaling events essential to the body. Overwhelming generation of ROS/RNS is likely to induce chronic inflammatory conditions that may lead to several deleterious effects in the cells. ROS/RNS interacts with important cellular targets, inducing changes in lipids, carbohydrates, proteins, and DNA. A schematic representation of several molecular events upregulated by ROS/RNS generated either by inhaled matter and/or produced by the activated inflammatory influx of cells during chronic inflammation promoting carcinogenesis is presented in Figures 2 and 3. Pulmonary diseases such as COPD, asbestosis, silicosis, and cancer are some of the major disorders that are induced by enhanced generation of ROS/RNS. It is apparent that increased levels of ROS/RNS are extensively involved in the mechanisms of chronic lung inflammation contributing to the development of lung cancer. A better understanding of the role of ROS/RNS in lung inflammation and cancer is likely to inspire new strategies for lung cancer prevention and treatment.

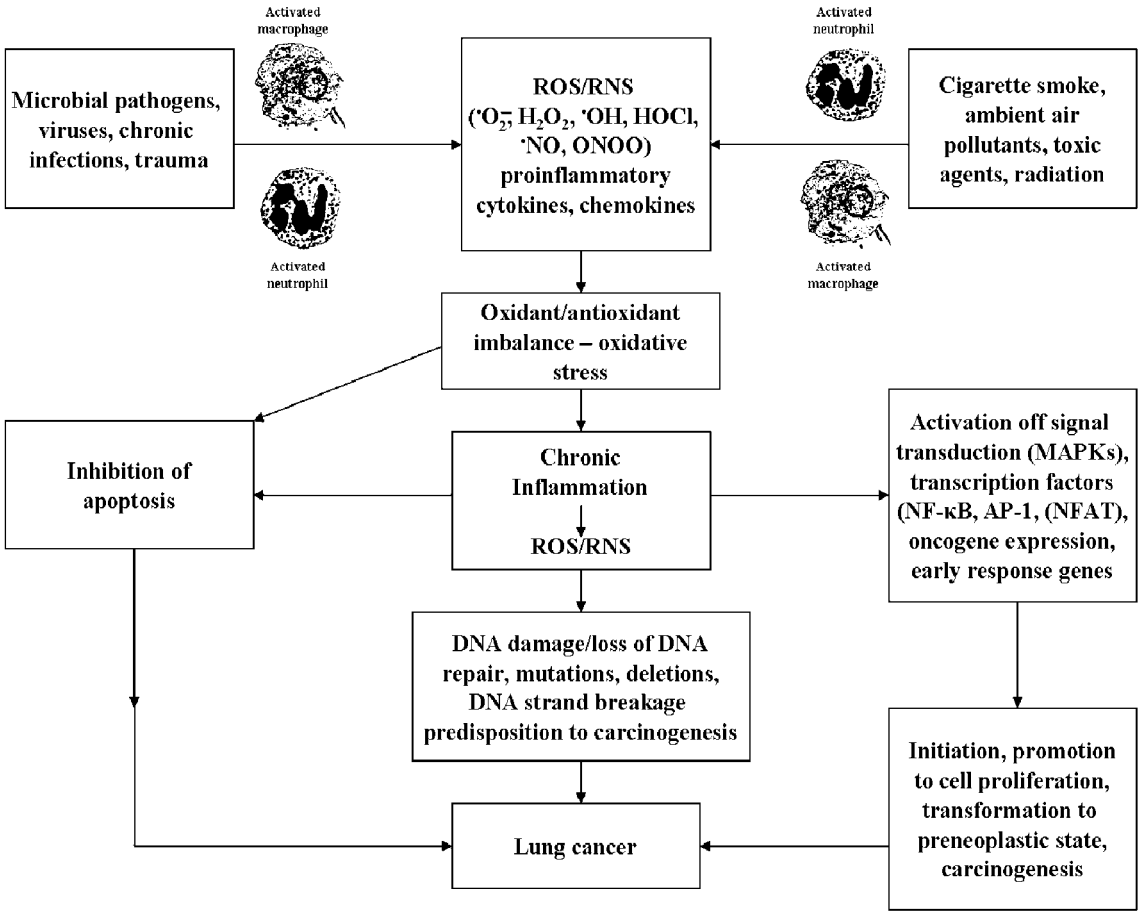

FIGURE 3. Schematic representation of major events leading to inflammation and cancer.

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