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Nuclear Factor-κB As Target for Chemoprevention AQ1

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ABBREVIATIONS

NF-κB, nuclear factor kappa B; IκB, inhibitor of NF-κB; CAPE, caffeic acid phenethyl ester; PBIT; S,S′-l,4-Phenylene-bis (1,2-ethanediyl) bis-isothiourea; PDTC, pyrrolidine dithocarbamate.

ABSTRACT

The process of tumorigenesis requires cellular transformation, hyperproliferation, invasion, angiogenesis, and metastasis. Several genes that mediate these processes are regulated by the transcription factor NF-κB. The latter is activated by

various carcinogens, inflammatory agents, and tumor promoters. The NF-κB, a transcription factor, is present normally in the cytoplasm as an inactive heterotrimer consisting of p50, p65, and IκBα subunits. When activated, NF-κB translocates AQ2 to the as a p50-p65 heterodimer. This factor regulates the expression of various genes that control apoptosis, viral replication, tumorigenesis, various autoimmune diseases, and inflammation. The NF-κB has been linked to the development of carcinogenesis for several reasons. First, various carcinogens and tumor promoters have been shown to activate NF-κB. Second, activation of NF-κB has been shown to block apoptosis and promote proliferation. Third, the tumor microenvironment can induce NF-κB activation. Fourth, constitutive expression of NF-κB is frequently found in tumor cells. Fifth, NF-κB activation induces resistance to chemotherapeutic agents. Sixth, several genes involved in tumor initiation, promotion, and metastasis are regulated by NF-κB. Seventh, various chemopreventive agents have been found to downregulate the NF-κB activation. All these observation suggest that NF-κB could mediate tumorigenesis and thus can be used as a target for chemoprevention and for the treatment of cancer. Agents, which suppress NF-κB activation, can suppress the expression of genes involved in carcinogenesis and tumorigenesis in vivo.

I. CARCINOGENESIS/TUMORIGENESIS

The process of tumorigenesis is a process that requires cellular transformation, hyperproliferation, invasion, angiogenesis, and metastasis. This process is activated by various carcinogens (such as cigarette smoke), inflammatory agents (such as TNF and H_2O_2), and tumor promoters (such as phorbol ester and okadaic acid) (1). Although initially identified as an anticancer agent (2), TNF has now been shown to be involved in cellular transformation (3), tumor promotion (4), and induction of metastasis (5–7). In agreement with these observations, mice deficient in TNF have been shown to be resistant to skin carcinogenesis (8). For several tumors,

TNF has been shown to be a growth factor (9,10). Like phorbol ester, TNF mediates these effects in part through activation of a protein kinase C pathway (11). Similar to TNF, other inflammatory cytokines have also been implicated in tumorigenesis (12,13). Thus, agents that can suppress the expression of TNF and other inflammatory agents have chemopreventive potential (14,15). Most carcinogens, inflammatory agents, and tumor promoters including cigarette smoke, phorbol ester, okadaic acid, H_2O_2, and TNF, have been shown to activate the transcription factor NF-κB.

II. CIGARETTE SMOKE AND CANCER

Cigarette smoke(CS) is a major cause of cancers of the lung, AQ4 larynx, oral cavity and pharynx, esophagus, pancreas, kidney, and bladder (16). Worldwide, one in seven or 15% (1.1 million new cases per year) of all cancer cases are attributable to CS, 25% in men and 4% in women. Recent estimates indicate that CS causes approximately 80–90% of lung cancer in the United States (17). Smoking during pregnancy and passive exposure to CS may increase the risk of cancer for children and adults (18–20). These estimates do not include the disease resulting from smokeless tobacco (taken orally or as snuff), which is a substantial cause of cancer mortality, particularly on the Indian subcontinent (21).

Tobacco smoke is a complex mixture containing at least 40 different carcinogens, which mediate tumor initiation and promotion. These carcinogens include nitrosamine, polycyclic aromatic hydrocarbons (PAH), aromatic amines, unsaturated aldehydes (e.g., crotonaldehyde), and some phenolic compounds (acrolein). The most potent carcinogenic agent contained in CS is the nitrosamine 4-(methylnitrosoamino) -l-(3-pyridyl) -l-butanone (NNK); formed by nitrosation of nicotine, it is thought to be an important etiological factor in tobacco–smoke related human cancers (22). The NNK is a site-specific carcinogen in that, irrespective of the route of administration, NNK has remarkable specificity for the lung (23). Because side-stream smoke often contains higher

amounts of NNK than mainstream smoke, passive exposure to CS has been suggested to be quite harmful (22). An enzyme 11 ??-hydroxysteroid dehydrogenase 1(11??-HSD1), which is involved in metabolism of endogenous steroids, is also responsible for the metabolism of NNK. Thus inhibition of 11??-HSD1 can increase the circulating levels of NNK by impairing its metabolism. Ethanol has been shown to be a potent inhibitor of 11??-HSD (24) and thus may increase the risk of lung cancer for active or passive smokers. An alcohol consumption and cigarette smoking have also been shown to increases the frequency of p53, a tumor suppressor gene, mutation in lung cancer (25).

Cigarette smoke has been shown to induce aryl hydrocarbon hydroxylase (AHH) activity, an activator of respiratory tract carcinogens of the PAH (e.g., benzo[a] pyrene) group (26), in human pulmonary macrophages (27) and in patients with smoking-associated malignant cancers (28). It has been postulated that individuals with high activity of oxidative enzymes (cytochrome P-450 enzymes) or a low activity of detoxifying enzymes (e.g., glutathione s-transferase and epoxide hydroxylase) may be at increased risk for cancer caused by CS (29). Low intake of dietary constituents with antioxidant properties such as carotene, vitamin C, and vitamin E further increases the cancer risk in smokers (30).

Lung tumors from nonsmokers exhibit elevated NAD(P)H:(quinone-acceptor) oxidoreductase (QAO) activity compared to normal tissue, but tumors from smokers show increases in tumor QAO (31). This could influence the response of these tumors to quinone drugs (commonly used to treat cancer) or toxic agents that are metabolized by QAO. Quinone anticancer drugs are activated to alkylating species by reduction to hydroquinone. Metabolism by QAO is responsible for the formation of alkylating species from doxorubicin (32) and other cytotoxic drugs (33).

Another possible mechanism by which CS can cause cancer involves the effects of PAH on the p53 gene. For instance, exposure of cells to benzo(a)pyrene adducts can induce the same mutation in p53 as is found in 60% of all lung cancers (34). Also exposure of cells to PAH and its metabolites results

in a rapid accumulation of the p53 gene product (35,36) through activation of a transcription factor, NF-κB (37).

III. EFFECT OF CIGARETTE SMOKE ON PULMONARY INFLAMMATION

Experimental epidemiological and clinical evidence indicates that CS is a primary risk factor for chronic obstructive pulmonary disease (COPD), which includes chronic bronchitis and emphysema. These two conditions result from obstruction of airflow and usually coexist. An increased proteolytic activity in the lung due to an imbalance between proteases, especially elastase and ??-1 protease inhibitor (1PI, an antielastase), has been suggested as a primary cause for COPD caused by CS. This occurs for three reasons. First, CS causes the generation of chemotactic factors (such as chemokines) (38), which recruit inflammatory cells (such as neutrophils and macrophages) to the lung, and these cells release proteolytic enzymes. Second, free radicals present in CS can either inactivate ??IP1 by oxidation of an active site methionyl residue present in the protein sequence or damage macromolecules to make them more susceptible to proteolysis. Third, components in CS can suppress elastin synthesis by inhibiting the cross-linking enzyme lysyl oxidase. Thus neutrophil recruitment, inactivation of protease inhibitors, and depressed tissue repair are considered responsible for the pathogenesis of CS-induced emphysema, although, only one in six smokers develop extensive COPD.

The inhalation of CS also results in inflammation of the pulmonary epithelia. Reactive oxygen intermediates (ROIs) are some of the most important effector molecules of acute inflammation. The inflammatory cell response to CS has been studied extensively either in cells harvested by bronchoalveolar lavage from cigarette smokers or smoke-exposed animals or in macrophages exposed to CS in vitro. Alveolar macrophages lavaged from smokers have increased oxidative metabolism compared to those in nonsmokers, and this leads to increased apoptosis of fibroblasts, which could be prevented

by oxidant scavenging agents. Thus oxidants generated by alveolar macrophages from smokers may facilitate tissue destruction (39).

IV. OXIDATIVE DAMAGE BY CIGARETTE SMOKE

Cigarette smoke has been implicated as major risk factor in COPD such as chronic bronchitis and emphysema, in chemical carcinogenesis, and in atherosclerotic arterial diseases. The mechanisms of the adverse biological effects of CS appear, in part, to include oxidative damage to essential biological constituents. The CS increases the number of phagocytes in the blood and lungs (40), decreases plasma levels of high-density lipoproteins (HDL) (41), and induces lipid peroxidation of LDL (42). Several plasma proteins have been shown to undergo modification by exposure to CS (43,44). In CS-bubbled buffers, H_2O_2 and hydroxyl radical were generated from aqueous extracts of tar (45,46). A superoxide radical was an intermediate in these reactions. Superoxide formed from CS impairs active oxygen generation from neutrophils.

V. COMPOSITION OF CIGARETTE SMOKE

Cigarette smoke is a complex mixture consisting of tarry particles of respirable size suspended in a mixture of organic and inorganic gases and containing more than 4000 chemical compounds. Inhaled mainstream, exhaled mainstream, and sidestream CS differ in composition. The CS contains two classes of free radicals, one in the gas phase and another in tar. The gas phase radicals consist of inorganic radicals (e.g., nitric oxide, NO) as well as organic radicals such as carbon- and oxygen-centered radicals. Nitric oxide is slowly oxidized to NO_2. It is estimated that there are approximately 10^{17} organic radicals per puff in gas phase smoke (Ref.46 and reference therein). Gas phase smoke is unstable and inactivates ??1PI. In contrast, tar radicals in the particulate phase are stable indefinitely and contain as many as 10^{18} free radicals

per gram, the major ones being quinone–hydroquinone complex. This complex is an active redox system capable of reducing molecular oxygen to produce superoxide, eventually leading to H_2O_2 and OH radicals. Tar also chelates metals, such as iron, that catalyze the decomposition of H_2O_2. An aqueous suspension of tar produces hydroxyl radicals and has been shown to cleave DNA. Many smokers have switched from high- to low-tar cigarettes. Though low tar cigarettes may expose the lungs to lower levels of carcinogens, they produce a higher burden of oxidants. Nicotine is the most important smoke component present in the blood of smokers, and it has a half-life of 2 hr. Nicotine affects the respiratory, cardiovascular, central nervous, and the endocrine systems. Another significant component of CS is Cd compounds, which have a long half-life, accumulate in the lungs, and induce acute inflammatory reactions in the lung and increased lung epithelial permeability.

VI. WHAT IS NF-κB?

The NF-κB represents a group of five proteins namely c-Rel, RelA (p65), Rel B, NF-κBl (p50 and pl05), and NF-κB2 (p52) (16). The NF-κB proteins are regulated by inhibitors of the IκB family, which includes IκBα, IκBβ, IκBε, IκBγ, Bcl-3, pl00, and pl05 (47). In an inactive state, NF-κB is present in the cytoplasm as a heterotrimer consisting of p50, p65, and IκBα subunits. In response to an activation signal, the IκBα subunit is phosphorylated at serine residues 32 and 36, ubiquitinated at lysine residues 21 and 22 and degraded through the proteosomal pathway, thus exposing the nuclear localization signals on the p50-p65 heterodimer. The p65 is then phosphorylated, leading to nuclear translocation and binding to a specific sequence in DNA, which in turn results in gene transcription. The phosphorylation of κBα is catalyzed by the IKK. The IKK consists of three subunits IKK-α, IKK-β, and IKK-γ (also called NEMO) (for references see Ref. 48). Gene deletion studies have indicated that IKK-β is essential for NF-kB activation by most agents (49). The kinase that AQ7

induces the phosphorylation of p65 is controversial, but IKK-β, protein kinase C, and protein kinase A have been implicated (17–19).

VII. RELEVANCE OF NF-κB TO CIGARETTE SMOKING

There are several reasons to believe NF-κB is a good target by which to examine CS-induced lung cancer development and its chemoprevention. First, benzo[a]pyrene, a component of CS, has recently been shown to activate NF-κB in lung adenocarcinoma cells (37) and in vascular smooth muscle cells (50). Second, CS is also a potent source of ROIs (44–46), which are required for NF-κB activation (47). Our laboratory and others have shown that antioxidants and overexpression of cells with antioxidant enzymes such as Mn superoxide dismutase or with γ-glutamylcysteinyl synthase (51–53) block NF-κB activation. Third, NF-κB activation has been implicated in chemical carcinogenesis and tumorigenesis (54,55). Fourth, CS has been shown to induce NF-κB-regulated chemokine genes in bronchial epithelium (Ref. 38 and references therein). Lastly our laboratory and others have shown that most chemopreventive agents suppress NF-κB activation (56–60).

VIII. WHY NF-κB IS IMPORTANT FOR CANCER?

The NF-κB has been shown to regulate the expression of a number of genes whose products are involved in tumorigenesis (20,21). These include antiapoptosis genes (e.g., cIAP, suvivin, TRAF, bcl-2, and bcl-xl) COX2; MMP-9; genes encoding adhesion molecules, chemokines, inflammatory cytokines and iNOS; and cell cycle regulatory genes (e.g., cyclin Dl) (22)). Thus, agents that can suppress NF-κB activation have the potential to suppress carcinogenesis and have therapeutic potential (21,23). The therapeutic role of phytochemicals in prevention and treatment of cancer has been indicated (24–26). Thus, plant-derived phytochemicals that could

suppress NF-κB activation by various carcinogens have been shown (Table 1). AQ10

IX. CHEMOPREVENTIVE AGENTS INHIBIT NF-κB ACTIVATION

Several agents that suppress carcinogenesis have been shown to block NF-κB activation. These include curcumin, green tea polyphenols, silymarin, and resveratrol (Fig. 1). Curcumin is AQ11 a polyphenol (diferuloylmethane) derived from the roots of *Curcuma longa*, and it inhibits both tumor initiation induced by BP and 7,12 dimethylbenz(a)anthracene and phorbol ester-induced tumor promotion (61–63). Both B[a]P and phorbol esters are potent activators of NF-κB. Curcumin has also been shown to suppress the expression of several genes involved in carcinogenesis including COX 2, lipooxygenases, and iNOS (64–67), also known to require NF-κB activation. Additionally, our laboratory has shown that curcumin blocks the TNF-induced expression of ICAM-1, VCAM-1, and ELAM-1, all NF-κB-regulated genes in endothelial cells, and needed for tumor metastasis (68). Our laboratory has also shown that curcumin suppresses the NF-κB activation induced by various tumor promoters in different cell types (56). Similarly, silymarin, derived from milk thistle (artichoks), has been AQ6 demonstrated to suppress carcinogenesis (69), and we have shown that this compound also inhibits NF-κB activation through blocking the phosphorylation and degradation of IκB (59). Resveratrol, derived primarily from grapes and peanuts, exhibits chemopreventive activity by inhibiting cellular events associated with tumor initiation, promotion, and progression (70). Our laboratory and others showed that resveratrol also blocks NF-κB activation and NF-κB-regulated expression of monocyte chemoattractant protein (MCP)-l (60,71). Thus, several of these examples suggest that suppression of NF-κB activation correlates with chemoprevention.

The epidemiological evidences also indicate that certain cancers (e.g., breast, prostate, colon, and lung) are more prevalent in the developed countries than in the developing

countries. It is most likely because of differences in dietary constituents (16,17). We propose that there are constituents of the every-day diet that regulate the activity of certain transcription factors such as NF-κB that plays a critical role in carcinogenesis.

X. CONCLUSION

Evidence presented above suggests that activation of NF-κB can lead to tumor cell proliferation, invasion, angiogenesis, and metastasis. Thus suppression of NF-κB in cancer cells may provide an additional target for prevention of cancer. The NF-κB blockers can also be considered for the therapy of cancer, perhaps in combination with chemotherapeutic agents or gamma irradiation.

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