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Biologic damage resulting from exposure to tobacco smoke and from radon: implication for preventive interventions

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Cigarette smoking and residential radon are, respectively, the first and second leading cause of lung cancer in the United States today. Of the approximately 157 000 lung deaths occurring in 2000, approximately 90% can be attributed to cigarette smoking and 30% of the lung cancer deaths among non-smokers can be attributed to residential radon exposure. Although dwarfed by cigarette related lung cancer, lung cancer among lifetime non-smokers is a leading cause of death in the United States, and many other countries, accounting for approximately 16 000 deaths per year in the US. Laboratory studies and epidemiological investigations, particularly those conducted in the past decade, are yielding evidence that tobacco smoke and radon may share important elements of lung cancer's pathologic mechanism(s). Lung cancer prevention among smokers, ex-smokers and lifetime nonsmokers can be enhanced as we learn more about the etiologic mechanism(s) of lung cancer resulting from these and other exposures including diet, non-malignant respiratory diseases, occupational exposures, and susceptibility-gene. In this article we review both laboratory and epidemiologic data that gives insight into the biologic damage done to the lung from these exposures.

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Introduction: lung cancer epidemiology

Lung cancer is now the leading cause of cancer death worldwide having surpassed gastric and colon cancer (Landis *et al.*, 1999). In 1998, the estimated worldwide incidence rates per 100 000 people were 37.5 for men and 10.8 for women, representing 18% of new cancers among men and 7.5% among women worldwide (Landis *et al.*, 1999). In the year 2000, 164 100 new cases of lung cancer were diagnosed in the United States, resulting in approximately 157 000 deaths (US Department of Health and Human Services, 2001). The 2001 Surgeon General's report on women and smoking concluded that 'about 90% of all lung cancer deaths among US women are attributed to smoking' (US Department of Health and Human Services, 2001). Similar attributable risk estimates were made for men in earlier reports by the Surgeon General (US Department of Health and Human Services, 1990).

Despite the acknowledged limitations of current risk estimates, the National Academy of Sciences BIER VI committee, concludes that indoor radon is the second leading cause of lung cancer (National Research Council, 1999). Although the attributable risk for radon is far less than that for smoking it may account for 21 800 lung cancer deaths per year (National Research Council, 1999). Recently, laboratory studies examining the mechanism of action by which tobacco smoke causes damage to the epithelium of the lung, and other studies examining the mechanism of radon damage in the lung, suggest important similarities between the two: the generation of free radicals and oxidative stress being an important early phase of both processes.

Other causes of lung cancer include diet, lung pathology resulting from pre-existing lung disease, occupational exposures and genetic factors (Blot and Fraumeni, 1996; Alavanja *et al.*, 1995). Asthma, emphysema, idiopathic pulmonary fibrosis, tuberculosis, chronic bronchitis and pneumonia have been associated with increased lung cancer incidence in non-smokers. A large multi-center, retrospective study showed that any history of chronic lung disease yielded an elevated risk ratio of 1.56 (Wu *et al.*, 1995), confirming earlier estimates of the same magnitude (Alavanja *et al.*, 1992). Although a comprehensive examination of the mechanism of action of all of these lung carcinogens is beyond the scope of this review, it is exciting to note that similarities among these agents and tobacco smoke and radon have been identified.

Continued research on the mechanism by which these exposures result in lung cancer is warranted for a number of reasons. One reason of particular importance may be enhanced disease prevention (i.e., through identification of susceptible individuals, developing techniques for early detection, improved nutrition and chemo-prevention). While lifetime avoidance of tobacco smoke exposure, or the earliest cessation of tobacco use for those who have started smoking is, by far, the most effective preventive action that could be taken, the question remains: will additional lung cancer risk

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reduction be achieved for smokers, ex-smokers and lifetime non-smokers by dietary manipulation or other pre-disease interventions?

A large number of epidemiologic studies suggest that fruit and vegetable consumption protects both smokers and non-smokers from the risk of lung cancer (reviewed in Ziegler *et al.*, 1996b; and selected studies by Candelora *et al.*, 1992; Maynes *et al.*, 1994; Nyberg *et al.*, 1998; Speizer *et al.*, 1999; Michaud *et al.*, 2000; Voorrips *et al.*, 2000), while some recent studies observed the protective effect primarily in lifetime non-smokers (Feskanich *et al.*, 2000) and others primarily in heavy cigarette smokers (Jansen *et al.*, 2001). If the majority of these studies showing a protective effect from fruit and vegetable consumption in the etiology of lung cancer among both smokers and non-smokers is true, the hypothesis that a similar pathologic mechanism for smokers and non-smokers would be enhanced. However, the epidemiologic studies of lung cancer among non-smokers have not yet attempted to determine if diets rich in fruits and vegetables are protective for all etiologically relevant exposures. Moreover, fruits and vegetables are a complex mixture of nutrients and it may be that specific nutrients are protective for some but not all exposures. More work is clearly needed to answer these important questions.

In this review we first briefly describe what we know about the carcinogenic properties of tobacco smoke and radon. We then systematically review selected literature which gives us insight into the damage done to the cells and sub-cellular components by these carcinogens that may be related to the disease process (a scheme that organizes our discussion of an etiologic pathway is presented in Figure 1). Finally we review the literature regarding the protective effect of diet and dietary supplements on lung cancer, along with the literature concerning the interaction of diet with polymorphic genes and lung cancer. Evaluating the pattern of protection afforded by different micro-nutrients and genes can yield additional mechanistic insights.

Characteristic of the carcinogens: tobacco smoke and radon

Tobacco smoke

Tobacco smoke is an aerosol. It is, therefore, composed of a gas phase and a particulate phase. The gas phases consists mainly of nitrogen, oxygen and carbon dioxide and is approximately 95% of the cigarette smoke by weight. The vapor-phase is easily separated from the particulate phase experimentally by glass fiber filters. Most of the carcinogens are contained in the particulate phase (also called tobacco tar) which contains at least 3500 compounds (Hecht, 1999b). The International Agency for Research on Cancer has identified 44 carcinogens in cigarette smoke for which there is 'sufficient evidence for carcinogenicity' in either laboratory animals or humans (IARC, 1986). Although, nicotine addiction is the reason people continue to smoke and find it very difficult to quit (Surgeon General, 1988), it is not a carcinogen itself (IARC, 1986).

Our knowledge of the mechanism by which these carcinogens in tobacco smoke cause lung cancer is not

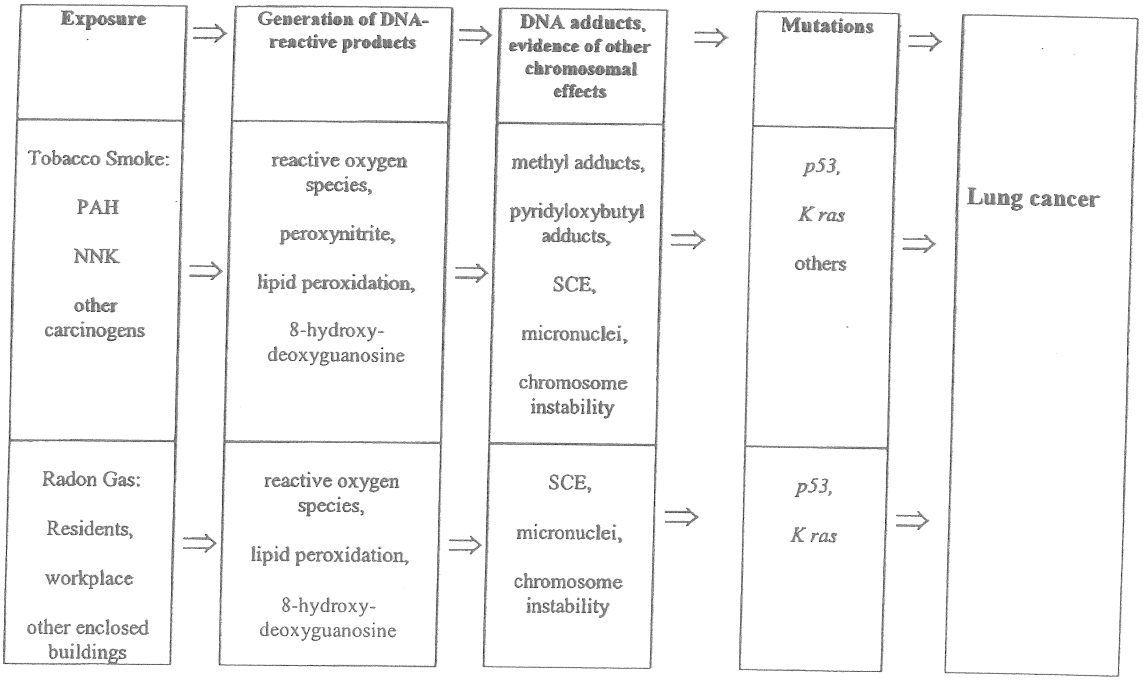

Figure 1 Scheme linking tobacco smoke and radon gas to induction of mutations to lung cancer (adapted from Hecht S. JNCI 1999; 91:1195.)

complete, but we have a wealth of information on their mechanism of action, much of it developed in the past decade (Hoffmann and Hoffmann, 1997). Two carcinogens for which we probably have the most toxicologic data, are polycylic aromatic hydrocarbons (PAHs) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-Butanone (NNK) (Hoffmann and Hoffmann, 1997). Both NNK and PAHs require metabolic activation to exert their carcinogenic effects. There are competing activation and detoxification pathways for carcinogens such as NNK and PAHs, that differ among individuals and will affect cancer risk (Hecht, 1999b). The carcinogenesis of cigarette smoke (particularly PAH and NNK) and radon gas are highlighted in subsequent sections.

Radon

Human exposure to radon (^{222}Rn) is ubiquitous, occurring as a result of seepage of this inert gas from uranium-containing rocks and soil into enclosed areas such as homes and underground mines. The carcinogenicity of radon is convincingly documented through epidemiologic studies of underground miners, all showing a substantial increased risk of lung cancer (Samet, 1989; National Research Council, 1999). In a pooled analysis of 11 miners studies, Lubin *et al.* (1994) reported RRs for lung cancer at 10 working level months as ranging from 1.2 to 6.1. Extrapolating these data using the linear no-threshold theory of radiation carcinogenesis to predict risk from residential exposure to ^{222}Rn, Lubin *et al.* concluded that, in the USA, exposure to radon progeny may account for 10% of all lung cancer deaths and 30% of lung cancer deaths in non-smokers (Lubin *et al.*, 1994), while an estimate from the National Academy of Sciences BEIR VI committee suggests 21 800 lung cancer cases annually resulting from radon exposure with uncertain bounds from 3000 to 33 000, making this the second leading cause of lung cancer in the United States (National Research Council, 1999).

The damage done to epithelial cells of the lung occurs when radiation interacts either directly with DNA in the cell nucleus or indirectly through the affect of free radicals (UNSCEAR, 2000). Recently, however, a number of *in vitro* studies of cells exposed to alpha-particle radiation gave evidence that more cells showed damage than were traversed by alpha-particles (Nagasawa and Little, 1992, 1999; Azzam *et al.*, 1998, 2000; Sawant *et al.*, 2001), a result of the so-called 'bystander' effect. Bystander effects result when irradiated cells emit signals (i.e., chemical by-products of radiation damage to the cell, presumably) that result in damage to nearby unirradiated bystander cells (Zhou *et al.*, 2000). Brenner *et al.* (2001) suggests that bystander effects can result in non-linear dose-response relations, and inverse dose-rate effects (i.e., greater than expected mutation rates for the same total dose delivered over a longer period of time). If true, estimates of lung cancer risk from domestic radon exposures derived from linear extrapolation models of miner data to low doses without accounting for dose rate effects, would underestimate risk by a factor of four. All investigators, however, are not convinced of the size of the bystander effect and its implied importance to population estimates of excess lung cancer risk from residential radon exposure (Little and Wakefield, 2001).

Oxidative stress

One of the very first steps in carcinogenesis of inhaled tobacco smoke is the generation of reactive oxygen species within the cells of the respiratory epithelium, resulting in oxidative stress to the cells (Pryor *et al.*, 1983). Molecular oxygen can pick up a single electron to form a number of intermediate, partially reduced oxygen species collectively termed reduced (or reactive) oxygen species (ROS). These include species such as peroxyl ($ROO^{\cdot}$) and alkoxyl ($RO^{\cdot}$) radicals and nitric oxides ($NO^{\cdot}$) (Church and Pryor, 1985). The generation of ROS is widespread in biological materials. Although there are beneficial actions that are brought about by oxygen-derived free radicals, much of the interest in these species is related to their potential to cause cellular damage (Church and Pryor, 1985). The most potentially hazardous, oxygen-derived radical is the hydroxyl radical ($HO^{\cdot}$) (Riley, 1994). ROS such as superoxides and hydrogen peroxide are relatively stable and their significance is essentially connected with their potential to give rise to hydroxyl radicals, which is the most reactive ROS (Church and Pryor, 1985).

The damage caused by hydroxyl radicals results from their biological interaction with structural and functional molecules, including lipids, nucleic acids, proteins and carbohydrates (Riley, 1994). Alterations in enzyme carrier or receptor functions have also been observed and altered enzymes may have important sequella (Williams, 2001). Oxidative damage to DNA-binding proteins may result in profound alterations in gene expression leading to changes in levels of certain oxidative stress-related proteins which may have protective functions or to the initiation of apoptosis (Carson and Ribeiro, 1993). The interaction of ROS, such as hydroxyl radical, with unsaturated lipids can result in lipid peroxidation (Slater, 1972). Peroxidation can also damage the cells by altering the structure and function of membrane lipids. The damage to the cell can be amplified in the course of the cascading, chain-branching of the peroxidation process. The cellular effects may, also, be exacerbated by the loss of compartmentation allowing leakage of enzymes (e.g. from lysosomes) or the collapse of diffusion barriers to electrolytes, transition metals and small molecules (Riley, 1994). Lipid peroxidation can, also, result in the production of compounds, such as 4-hydroxynoneal, that can diffuse through the nuclear membrane and cause alteration in the structure and function of nucleic acids and protein (Riley, 1994; Bartsch *et al.*, 1997). DNA damage by hydroxyl radicals may lead either to mutation or to major chromosomal derangement which may be cytotoxic to proliferating cells (Riley, 1994).

Cigarette smoke

Cigarette smoke contains large amounts of reactive oxygen species that are known to be present in both gas-phase smoke and cigarette tar (i.e., particulate phase) (Pryor *et al.*, 1983; Church and Pryor, 1985; Leanderson and Tagesson, 1992). Gas-phase smoke contains up to 500 p.p.m. nitric oxide and nitrogen dioxide, both of which are short-lived radicals (Cueto and Pryor, 1994). The radicals in tar are longer-lived semiquinones (Pryor, 1997). Aqueous-extracts of cigarette tar (ACT) contain a low-molecular weight quinone-hydroquinone-semiquinone system which is capable of producing superoxides and hydrogen peroxide and the hydroxyl radical which are all potent oxidants (Pryor, 1997). *In vitro* studies have demonstrated that cigarette smoke can generate hydrogen peroxide and hydroxylate deoxyguanosine residues in isolated DNA (Leanderson and Tagesson, 1992) as well as in cultured human lung cells (Leanderson and Tagesson, 1992). ACT solutions can initiate lipid peroxidation, oxidize proteins (Evans *et al.*, 1991; Evans and Pryor, 1994) and nick DNA (Stone *et al.*, 1994). *In vivo*, oxidative damage to leukocyte DNA (Degan *et al.*, 1995; Asami *et al.*, 1996) and to sperm DNA (Fraga *et al.*, 1996) has been found to be elevated in smokers compared with non-smokers and the production of reactive oxygen species found in leukocytes was higher in smokers than non-smokers (Kalra *et al.*, 1991).

Oxidative modification of DNA includes a variety of base oxidations (Demple and Harrison, 1994). C-8 hydroxylation of the guanine base in DNA is rapidly and almost completely repaired (Teebor *et al.*, 1988; Breimer, 1991), mainly by an excision repair mechanism (Bessho *et al.*, 1993). The excised oxidized nucleoside 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG*) is water-soluble and readily excreted in the urine without further metabolism and its rate of urinary excretion has been validated as a biomarker of the rate of oxidative DNA modification (Loft and Larsen, 1996; Loft *et al.*, 1995). In population-based studies, it has been demonstrated that smokers excrete approximately 35 to 50% more 8-oxodG in urine than non-smokers (Loft *et al.*, 1992, 1994).

The relevance of 8-oxodG as a biomarker of oxidative DNA modification is supported by the observation that the guanine base is a major target of oxidative DNA damage (Aruoma *et al.*, 1991; Dizdaroglu, 1991) and that the oxidation of guanine residues in DNA is mutagenic (Kuchino *et al.*, 1987). The relation of oxidative DNA leads to GC>T transversions (Cheng *et al.*, 1992), as found in the activated K-*ras* oncogene (Higinbotham *et al.*, 1992) and in the tumor suppressor gene of human cancers (Hollstein *et al.*, 1991). Recently, it has been shown that benzo[a]pyrene, a carcinogenic constituent of tobacco smoke, causes strong and selective adduct formation at guanine positions, including codon 248 of the *p53* gene, which is a major mutational hotspot in human lung cancer (Denissenko *et al.*, 1996). Interestingly, treatment with oxidants has been shown to cause similar mutations in codons 248–250 of *p53* (Hussain *et al.*, 1994).

Prieme *et al.* (1998) found a statistically significant effect of smoking cessation for 4 and 26 weeks on the urinary excretion rate of the DNA repair products between the control group and the smoking cessation group. The study gives direct evidence that smoking induces oxidative DNA modification.

Radon

Alpha particles like those emitted from radon decay have a high linear energy transfer (LET) depositing energy in a spatially concentrated fashion. Approximately 80% of the energy if ionizing radiation deposited in cells results in the ejection of electrons from water (Adams, 1986; Riley, 1994).

$$H_2O \rightarrow H_2O^+ + e^-$$

Reactions with the surrounding water then results in the production of several reactive species: e^-_{aq}, $HO^\cdot$, $H^\cdot$, H_2, H_2O_2 (Adams, 1986; Riley, 1994). These reactive oxygen species interact with themselves and with surrounding molecules. When oxygen is present, superoxide radicals are formed (Adams, 1986; Riley, 1994).

$$e^-_{aq} + O_2 \rightarrow O^\cdot{}_2{}^-$$

$$H^\cdot + O_2 \rightarrow O^\cdot{}_2{}^- + H^+$$

Hydrogen peroxide is generated from the superoxides. The time scale of this reaction (dismutation) is dependent on the pH (Adams, 1986; Riley, 1994).

$$2O^\cdot{}_2{}^- + 2H^+ \rightarrow O_2 + H_2O_2$$

Superoxide and hydrogen peroxide are relatively stable compared to most primary radical products. While most primary radical products persist for only 1 ms, superoxides and hydrogen peroxide exist for longer periods and can therefore diffuse to more distant sites. The hydroxyl radicals formed from ROS cause biologic damage which is an indirect, but important, source of radiation injury (Riley, 1994).

One result of oxidative stress is cellular damage by hydroxyl radical attack. The amount and rate of hydroxyl radical generation from ROS is controlled partly by the availability of reducing systems capable of reducing (or 'activating') superoxides or hydrogen peroxide. The cellular antioxidant status determines the intracellular concentration of ROS. It has been shown that the effects of H_2O_2 resemble those of ionizing radiation. Cytotoxicity of H_2O_2 is a function not of concentration but of total amount present per cell

(Jonas and Riley, 1992). Cells exhibiting high levels of superoxide dismutase (SOD), catalase and peroxidase activity are relatively less venerable to the secondary effects of radiation.

Although the mechanism(s) by which high-LET alpha particles emitted inhaled radon and radon progeny cause cancer is not known. Lehnert and Goodwin (1997), have shown that a short-lived, sister chromatid exchange factor(s) were generated in alpha-irradiated culture medium containing serum in the absence of cells. Lehnert has also demonstrated that the activity of this unidentified factor was promptly inhibited by superoxide dismutase. Exposing fibroblast to alpha particles produced a more persistent SCE-inducing factor(s), which can survive freezing-thawing, is heat labile and is inhibited by superoxide dismutase. Lehnert's findings suggest the medium-derived factor is a free-radical mediated process that involves the formation of superoxide anions. At least two general mechanisms for the production of superoxide anions ($O^{\cdot -}_2$) in alpha-irradiated, cell-free medium are possible. With one, $O^{\cdot -}_2$ could be expected to be generated upon the interaction of molecular oxygen with either electrons (e^-_{aq}) that were ejected from water by the ionizing radiation or with the radiolytic product $H^{\cdot}$

$$e^-_{aq} + O_2 \rightarrow O^{\cdot}{}_2{}^-,$$

$$H^{\cdot} + O_2 \rightarrow O^{\cdot}{}_2{}^- + H^+$$

A second potential source of $O^{\cdot}{}_2{}^-$ could arise from products of lipid peroxidation, perhaps initiated by hydroxyl radicals. Chamulitrat *et al.* (1991) and De Kok *et al.* (1994) suggest that hyperoxides of polyunsaturated fatty acids can be reduced to form alkoxyl radicals that react with oxygen after intramolecular rearrangement and release superoxide radical ions. von Sonntag (1994) suggests superoxide anions are sufficiently stable to allow diffusion within cells and therefore may generate DNA-damaging hydroxyl radicals from these radicals or generate hydrogen peroxide. Since Lenhert's SCE-inducing factor derived from irradiating fibroblasts from cells survives freezing/thawing cycles and is heat labile, it suggests that the factor may be proteinaceous (Lehnert and Goodwin, 1997).

In an elegant study, Wu *et al.* (1999) demonstrated that 8-OHdG (i.e., 8-hydroxy-deoxyguanosine), a reliable marker for oxidative DNA damage in mammalian cells (Ames, 1989; Yarborough *et al.*, 1996), was generated in Chinese hamster ovary (CHO) whose cytoplasm was irradiated with between 4 and 8 alpha particles. The maximum concentration of 8-OHdG among irradiated cells was observed 5 min after irradiation and the elevated levels were no longer in evidence 45 min after irradiation.

The data from these studies confirm the generation of ROS and oxidative stress to cells irradiated with alpha particles *in vitro*.

It might be expected, therefore, that we would find elevated concentrations of 8-oHdG in the urine of those highly exposed to radon and radon progeny since an oxidative modification of DNA is suggested similar to that observed for smokers. The fact that this has not been observed among miners highly exposed to radon does not necessarily mitigate the importance of this mechanism in radon carcinogenesis, rather it suggests the total dose of radon is small compared to the dose of oxidative species in mainstream tobacco smoke.

Later damage: DNA adducts/clastogenic effects/sister chromatid exchange/mutations

Cigarette smoke

Recent developments have produced more reliable dosimetric techniques to quantify PAH–DNA adducts in human tissue (Kriek *et al.*, 1998). Early *in vitro* studies show that the interaction of highly reactive (+)-anti-B[a]P diolepoxide with DNA and polynucleotides reacts mainly with guanine, with the C-10 carbon of B[a]p becoming linked to the exocyclic 2-amino group (Osborne *et al.*, 1976; Jeffrey *et al.*, 1976). A number of epidemiologic and laboratory studies have shown smoking-related DNA adducts in human lung tissue and in white blood cells to be good dosimetric exposure markers. PAH–DNA adducts reflect individual response to exposure via lifestyle, occupation or ambient air pollution, and possibly the modulation of exposure is influenced by genetic factors and micronutrients (Grinberg-Funes *et al.*, 1994; Mooney *et al.*, 1995, 1997; Bartsch *et al.*, 1991, 1995b). Recent smokers among cancer patients had significantly higher induced lung CYP1A1-related enzyme activity compared to non-cancer patients who were also smokers (Bartsch and Hietanen, 1996; Bartsch, 1996).

In smokers and ex-smokers a highly significant positive correlation was found between pulmonary CYP1A1-induced enzyme activity and lung B[a]P-DNA adducts ($r=0.91$, $P=0.01$), and the B[a]P-DNA adduct levels accounted for approximately 20% of the total aromatic DNA adducts detected (Vahakangas *et al.*, 1985).

The *p53* tumor suppressor gene is commonly mutated in human cancer, with mutations most commonly occurring in exons 5–8 (Hussain and Harris, 1998). Smoking is most frequently associated with G:C–>T:A transversions in *p53* mutations (Hussain and Harris, 1998; Westra *et al.*, 1993).

A positive association between lifetime tobacco use and the frequency of *p53* and G–>T transversions on the nontranscribed DNA strand have also been observed (Westra *et al.*, 1993). In a review of the literature Hecht (1999b), concluded that while carcinogens in tobacco smoke are responsible for a substantial percentage of the G mutations in the *p53* gene from human lung cancers, the assignment of these mutations to specific carcinogens is still speculative.

K-*ras* mutations in codon 12 are found in 24–50% of human primary adenocarcinomas of the lung but are infrequently observed among other tumor types (Kennedy *et al.*, 1996). These mutations are more

frequent in smokers and ex-smokers than in non-smokers, suggesting that they may be induced by direct reaction with an activated tobacco smoke carcinogen (Kennedy *et al.*, 1996).

Radon

Prior to the 1990s the biologically significant damage to the epithelial cells of the lung from radon was believed to be the result of an alpha particle hitting the nucleus and causing double strand breaks in the chromosome. Some fraction of these chromosome breaks were presumed to result in permanent mutations important to the carcinogenic process. During the 1990s however, the development of precision particle micro beams made it possible to target known numbers of alpha particles to exact locations within the cell. This technological development resulted in experiments that provided convincing evidence that extra-nuclear targets are the significant loci of alpha particles interacting with the cell. The interaction results in chemical by-products in the cell cytoplasm similar to those produced from exposure to chemical carcinogens such as those contained in tobacco smoke. While the mechanism by which alpha particles cause lung cancer has not been elucidated, a variety of genetic lesions, including chromosomal damage, gene mutations, induction of micronuclei, and sister chromatid exchange (SCE), have been associated with the DNA-damaging effects of alpha particles (Bartsch *et al.*, 1995a; Kennedy *et al.*, 1996; Brooks *et al.*, 1990; Deshpande *et al.*, 1996; Nagasawa and Little, 1992; Wu *et al.*, 1999; Hei *et al.*, 1997, 1998). Emerit *et al.* (1995) found that the classtogenic properties of blood from personnel accidently irradiated at the Chernobyl nuclear power regressed and nearly completed disappeared after treatment with the extract of Ginkgo biloba, which is known for it superoxide scavenging properties.

Using a precision charged particle micro beam, Wu *et al.* (1999) showed recently that irradiation of cellular cytoplasm with either a single or exact number of alpha particles resulted in mutation in the nucleus while inflicting minimal toxicity, and that free radicals mediate the mutagenic process. Hei *et al.* (1997) determined that for alpha-particle doses of equal toxicity, that is approximately 90% cell survival, irradiation of the cytoplasm resulted in seven times more mutations than irradiating the nucleus. Of additional mechanistic interest is that treatment of this *in vitro* system for 10 min before and 10 min after alpha-irradiation of the cytoplasm with dimethyl sulphoxide (DMSO) resulted in significant suppression of mutation to near background levels, while treatment of the *in vitro* system with buthionine-S-R-sulphoximine (BSO) for 18 h prior to alpha-irradiation, which reduced the intracellular glutathione content to $<5\%$ of the level in control cells, increased mutations by 4–5-fold. The doses of both DMSO and BSO used in this experiment were previously shown to be nontoxic and non-mutagenic in mammalian cells (Hei *et al.*, 1998).

Because DMSO is a well-established free-radical scavenger, particularly for hydroxyl radicals (Kennedy and Symons, 1997), one would expect $OH^{\cdot}$ to be an integral part of the initial signal. However, $OH^{\cdot}$ is short-lived and can only diffuse approximately 4 nm, whereas Wu's irradiation were 8000 nm from the nucleus (Roots and Okada, 1972). Free radical induction of lipid peroxidation might be one possible explanation. Another possible mechanism might be that direct or indirect alpha-particle effect on the mitochondrial DNA with the generation of organic radicals such as peroxynitrite ions (Wei, 1998; Lenaz, 1998).

Prise *et al.* (1998) reported that a single human fibroblast irradiated with five alpha particles from a micro beam induced a significant increase in micronuclei among neighboring cells, although no mechanistic explanations were provided in this study as to how a single irradiated cell mediated a bystander response. In an experiment by Zhou *et al.* (2000), it was shown that irradiation of 20% of randomly selected Al cells with 20 alpha particles each resulted in a mutation fraction that is threefold higher than expected, assuming no bystander effect (Hickman *et al.*, 1994). This study provides clear evidence that irradiated cells can induce a bystander mutagenic response in neighboring cells not directly traversed by alpha particles and that cell–cell communication processes play a critical role in mediating the by-stander phenomenon (Zhou *et al.*, 2000).

There is now evidence beginning to emerge (Hei *et al.*, 1997) suggesting that the mutation spectra resulting from alpha particles traversing a nucleus is substantially different to the spectra resulting from cytoplasmic traversal. Mutations resulting from cytoplasmic traversal resemble the spontaneously occurring mutations thought to arise from endogenous reactive oxygen species (Ross and Goncharova, 1998), while the mutational spectra from nuclear-traversals are very different. An earlier study by Hickman *et al.* (1994) in which immortalized rat lung epithelial cells were used, showed increase expression of the *p53* tumor suppressor gene in the bystander cells of those irradiated with alpha particles. The epidemiological data concerning the specificity of mutational spectra observed in human population highly exposed to radon has not been consistent. Two investigations among underground miners (Vahakangas *et al.*, 1992; Taylor *et al.*, 1994) and one among residents of a nation-wide population-based investigation of residential radon (Yngveson *et al.*, 1999) indicated unusual mutation patterns in the *p53* gene.

Evidence of mechanism from studies of vegetable consumption and genes

The literature concerning the protective effects of dietary fruit and vegetable for lung cancer risk is extensive but not always consistent. Nonetheless, in aggregate this literature does shed some additional

light on the potential mechanism of action of tobacco smoke and alpha radiation from radon.

Results from more than 30 case–control and cohort studies indicate that people who eat more vegetables and fruit have a lower risk of lung cancer than those who eat fewer such foods and the protective effect is observed in current smokers, ex-smokers and never-smokers (Steinmetz and Potter, 1991; Ziegler *et al.*, 1996b). The mechanism by which vegetables and fruit protect the lungs and other organ systems from the harmful effects of certain carcinogens is not known with certainty. In fact, due caution needs to be exercised in interpreting the beneficial effects of vegetable consumption as the result of a single nutrient. The hypothesis that beta-carotene was principally responsible for the protective effects of fruits and vegetables, for example, was largely refuted by the results from a large intervention control trial which strongly suggests that excess lung cancer incidence and overall mortality may result from beta-carotene supplementation (Albanes, 1999).

Mutagenesis and carcinogenesis in animal studies have been inhibited by a large number of compounds from vegetables and fruits, including carotenoids, polyphenols, thiols, trace metals, terpenes, tocopherols, and degradation products of glucosinolate (Byers and Perry, 1992; Krinsky, 1993; Bendich, 1994; Zhang *et al.*, 1992). Antioxidant micronutrients in fruits and vegetables include the carotenoids themselves, vitamin E (a fat-soluble vitamin known to prevent lipid peroxidation), vitamin C (a water soluble vitamin), and selenium (a trace mineral essential for several enzymes, including gluthiathione peroxidase) (Albanes, 1999). In addition to the large number of antioxidants contained in vegetables and fruit, the micronutrient antioxidants can have a number of different physiological effects on the cell. Carotenoids, for example, are not only antioxidants and free radical quenchers (Ziegler *et al.*, 1996a), but also modulate the immune system (Steinmetz and Potter, 1991) and affect gap junction communication (Byers and Perry, 1992).

In a recent study by Michaud *et al.* (2000) alpha-carotene and lycopene consumption were significantly associated with a lower risk of lung cancer in a pooling of the Nurses Health Study (NHS) and the Health Professional Follow-Up Study (HPFS). Among lifetime non-smokers, a significant 63% lower risk of lung cancer was observed for the highest *vs* the lowest quintile of alpha-carotene consumption, while among current smokers, a significant inverse association was observed for lycopene consumption and lung cancer risk, but not for the other carotenoids. Similar results were observed for a cohort from Finland, where alpha-carotene consumption was inversely associated with lung cancer risk and non-significant but suggestive reductions in risk were observed for beta-carotene, lutein, and beta-cryptoxanthin (Knekt *et al.*, 1999). More evidence for the protective effect of carotenoids were also found in all three case–control studies that examined specific carotenoids. Alpha-carotene was associated with a significant reduction in risk in two of the three studies (le Marchand *et al.*, 1993; Ziegler *et al.*, 1996a) and a non-significant reduction in risk in the third (Garcia-Closos *et al.*, 1998).

In vitro studies show that lycopene is the most efficient carotenoid scavenger of free radicals (Di Mascio *et al.*, 1989) and lycopene is more efficient than beta-carotene is at preventing cell membrane damage from nitrogen dioxide radicals found in cigarette smoke (Bohm *et al.*, 1995). Some have speculated that the in addition to the known properties of these compounds just enumerated, it may be possible that carotenoids interact with each other to prevent oxidant injury to the cells of the lung (Mortensen *et al.*, 1997).

A number of laboratory and epidemiological studies have shown lung cancer risk reduction to be particularly associated with Brassica vegetables and possibly the interaction of metabolites of Brassica vegetables (i.e., isothiocyanates) and genetic polymorphisms. The consistency of several recent studies has further implications for a carcinogenic mechanism. Brassica vegetables, including cabbage, kale, broccoli, Brussels sprouts, and cauliflower contain a relatively high content of glucosinolates. Glucosinolates undergo enzymatic hydrolysis to isothiocyanates and indoles. Isothiocyanates have been shown to be effective inhibitors of tumorigenesis in animal models (Chung, 1992; Hecht, 1999a). Several mechanisms have been proposed for this effect.

Isothiocyanates are known to interfere with the metabolic activation of procarcinogens in tobacco smoke by cytochrome P450s (i.e., phase I activation enzymes) and also by enhancing the glutathione-S-transferases detoxification system (i.e., phase II detoxification enzymes) resulting in the beneficial metabolism in the chemical carcinogenesis of exogenous agents (Di Mascio *et al.*, 1989; Bohm *et al.*, 1995; Mortensen and Skibsted, 1997; Chung, 1992; Hecht, 1999a). Isothiocyanates may also inhibit carcinogenesis by inducing apoptosis (Yu *et al.*, 1998) or protecting against oxidative damage (Fahey and Talalay, 1999; van Poppel *et al.*, 1999). More information of these systems are necessary, but recently, phenethyl isothiocyanates (PEITC) (a chemo preventive agent against lung cancer induced by the tobacco-specific lung cancer 4-(methylnitrosamino)-1-(3-pyridyl-1-butanone) (NNK) in rats and mice) was shown to help detoxify NNK in humans. This was shown in a small controlled trial of 11 smokers who ate watercress (nasturtium officinale) with a minimum PEITC consumption averaging 19–38 mg/day. The metabolic activation of NNK into carcinogenic metabolites was inhibited as observed by increased levels of detoxified metabolites excreted in urine (Hecht *et al.*, 1995).

Gluthione-S-tranferases (GST) are a family of enzymes that detoxify reactive electrophiles such as epoxides (van Lieshout *et al.*, 1998; Verhagen *et al.*, 1997; Ketterer, 1998). A very common deletion in the *GSTM1* and *GSTT1* genes, creates a variant polymorphism that eliminates the respective enzyme activity in this detoxification enzyme system. Deficiency in

GSTM1 and *GSTT1* activity has been associated with a small increase in lung cancer risk in some but not all studies (Houlston, 1999). Of interest to our focus on the mechanism of lung cancer in smokers and non-smokers, is that the GST family includes enzymes that conjugate isothiocyanates with glutathione resulting in the elimination of isothiocyanates from the cell (Kolm *et al.*, 1995). Since the protective effective of isothiocyanates could be decreased by GST, the interaction of isothiocyanates and GST has been the focus of three epidemiologic studies to date. In a case–control study of Chinese women living in Singapore, Zhao *et al.* (2001) found that weekly consumption of isothiocyanates above the mean concentration in the controls was associated with a reduction in risk that was larger in smokers than non-smokers (i.e., OR = 0.31 (0.10–0.98) in smokers; OR = 0.70 (0.45–1.11) in non-smokers). Of additional interest is the observation that, among non-smokers, in the subgroup with GSTM1-null and high intake of isothiocyanates the lung cancer risk was reduced by 50% and this effect was not altered by controlling for environmental tobacco smoke or the consumption of other fruits and vegetables. The key findings of this report have been previously been observed in two other groups. London *et al.* (2000) observed that among Chinese men living in Shanghai, study participants with measurable urinary isothiocyanates concentration had a significantly lower risk of lung cancer than those with non-detectable concentration in their urine. Moreover, this protective effect was primarily limited to individuals with the null genotypes of *GSTM1* and *GSTT1*. Spitz *et al.* (2000) found a similar effect studying a population in the United States, namely, that a combination of low isothiocyanates consumption and the null genotypes of *GSTM1* and *GSTT1* resulted in the highest risk of lung cancer among smokers. The combination of these three well executed studies in three widely separated populations confirms an important interaction between dietary isothiocyanates and the *GSTM1* and *GSTT1* genotypes and lung cancer risk. Although even more work needs to be done to explore this complex relationship, the effect seems to be consistent for both smokers and non-smokers. It would be of particular interest to our focus on the mechanism of action of tobacco smoke and radon to conduct a similar study in a population where radon exposure and other lung cancer risk factors were well documented.

Conclusions

Although we do not know the complete mechanism of action for any lung carcinogen at the present time, tremendous progress has been made, particularly in the last decade. The increased pace of progress may be attributed to technological advancements in a number of areas including micro beam dosimetry, which makes it possible to target specific sites within a cell with precise doses of alpha particles, and advances in molecular biology and analytical chemistry which make it possible to detect both the composition of DNA adducts with ever greater sensitivity and the presence of oxidative radicals of biologic importance, some of which exist for mere nanoseconds.

Using these tools, in *in vitro* studies and epidemiological studies, we are now finding striking similarities between the initial biological damage caused by exposure to tobacco smoke and exposure to radon gas (Table 1). Rather than causing carcinogenically important damage to the cell only when the alpha particle interacts with the nucleus, a number of studies found that chemical by-products from the cytoplasmic interaction with alpha particles can cause biologically important alterations in the DNA of the target cell and in adjacent, non-traversed cells. These events are similar to those resulting after pro-carcinogens in tobacco smoke are activated by phase I enzymes. Some, if not most, of the initial by-product is reactive oxygen species. The cell damage resulting from this oxidative stress has been measured in epidemiologic studies of smokers and in *in vitro* studies of cells exposed to alpha particles from radon. Subsequent to the initial interaction between the alpha particle and the cytoplasm, evidence suggests lipid peroxidation may be involved in a next step of the chain of events. Evidence of deleterious peroxidation has been observed in both smokers and non-smokers. The benefit of a diet high in watercress in reducing urinary markers of oxidative stress in a small controlled study of smokers suggests the possibility of chemo-preventative action. Similar studies have not been conducted on populations exposed to high radon gas concentrations or other suspected lung carcinogens.

At a later stage in this process DNA damage in the form of sister-chromatid exchange and mutations have been observed for both smokers and those exposed to radon gas. Although some similarities in the increased frequency of p53 mutations have been identified in both groups, the data is inconclusive and there continues to be even more uncertainty in the loci of mutational hot-spots associated with these exposures.

Table 1 Biological damage to lung epithelial cells resulting from exposure to alpha-radiation and tobacco smoke

Observed biological effect	*Tobacco smoke*	*Alpha-radiation from radon*
Generation of ROS	Substantial evidence	Substantial evidence
Oxidative stress (i.e., oxidative DNA damage)	Substantial evidence	Substantial evidence
Sister chromatid exchange	Substantial evidence	Substantial evidence
DNA adducts	Substantial evidence	Little evidence
p53 mutations	Substantial evidence	Some evidence, inconsistent
k-ras mutations	Substantial evidence	Some evidence, inconsistent
Other mutations	Substantial evidence	Some evidence
Genetic instability	Substantial evidence	Some evidence
Evidence of diet–environment interaction	Growing evidence	Indirect, suggestive evidence from non-smokers only

This area of research is undergoing rapid development and clarification is anticipated.

Data from a growing number of large epidemiological studies have suggested the benefit of diets high in fruits and vegetables. The benefit is, at least in part, thought to be associated with carotenoids, isothiocyanates, selenium or a combination of these compounds. While we do not know the entire mechanism by which these benefits are imparted, their antioxidant properties seem to play an important role. Although there is some disagreement as to whom may derive the greatest benefit, high intake of vegetables and fruit seems to be associated with a benefit to smokers, ex-smokers and lifetime non-smokers in a preponderance of the published studies. At least three recent studies conducted in different populations have shown isothiocyanates from cruciferous vegetable consumption significantly protects against lung cancer and the benefit of isothiocyanate intake is modulated by the *GSTM1* and *GSTT1* genes. These effects have been shown among both smokers and non-smokers. While no studies such as these have been conducted in populations with known radon gas exposures, they would be of mechanistic interest.

In summary, we observe in both laboratory and epidemiologic studies that there are many similarities between the pathologic mechanism observed among those exposed to tobacco smoke and those exposed to radon gas. There is also growing evidence that micronutrients that are associated with a reduction in lung cancer risk among smokers, also reduce the risk in non-smokers and the subtle interaction between micronutrients and the *GSTM1*/*GSTT1* genotypes may also be observed in both smokers and non-smokers. While the totality of these studies do not prove a common pathologic mechanism, they suggest similar preventative action can be taken at this time, while we continue to gain biologic insight into lung cancer etiology.

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