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Increased Urinary 8-Isoprostaglandin F₂α Is Associated With Lower Plasma Selenium Levels and Lower Vegetable and Fruit Intake in an Asbestos-Exposed Cohort at Risk for Lung Cancer*

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(CHEST 2004; 125:83S)

Abbreviation: 8-EPG = 8-isoprostaglandin F₂α

Oxidative damage is a putative mechanism in the pathogenesis of asbestos-related lung diseases, including lung cancer. We previously have shown that the lipid peroxidation product 8-isoprostaglandin F₂α (8-EPG) in urine was positively associated with years of asbestos exposure and smoking status in an asbestos-exposed cohort. We analyzed the influence of diet (*ie*, fruit and vegetable intake) and plasma

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This research was supported by National Cancer Institute grants K-23 CA84034, R-01 CA84059, and M01-RR00051.

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selenium levels (a marker of selenium status) on urinary 8-EPG levels in this population.

MATERIALS AND METHODS

Seventy-nine asbestos-exposed construction trades workers completed a seven-item food frequency questionnaire that was validated for assessing daily fruit and vegetable intake. An administered questionnaire elicited age, smoking status, and asbestos exposure. After solid phase extraction, we measured urinary 8-EPG concentrations using an enzyme-linked immunosorbent assay kit. Plasma selenium levels were determined by a fluorometric procedure.

RESULTS

We found an inverse association between urinary 8-EPG levels, and both daily fruit/vegetable intake and plasma selenium levels. This association remained significant after controlling for age, current smoking status, and duration of asbestos exposure. The regression model showed a strong interaction between smoking status and self-reported daily fruit/vegetable consumption. For 16 current smokers and 63 former smokers or never-smokers, the standardized regression coefficients were markedly different at $\hat{\beta} = -0.67$ ($p = 0.01$) and, $\hat{\beta} = -0.23$ ($p = 0.06$), respectively.

CONCLUSION

The oxidant injury marker urinary 8-EPG is significantly higher in asbestos-exposed workers reporting low fruit/vegetable intake, particularly in smokers. Low plasma selenium levels are also predictors of higher 8-EPG levels. These findings may have implications for preventive interventions such as dietary modification and selenium supplementation in cohorts that are at risk for lung cancer. The association between urinary 8-EPG levels and biomarkers of lung cancer risk in sputum is currently under investigation in this cohort.

p53*

At the Crossroads of Molecular Carcinogenesis and Molecular Epidemiology

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(CHEST 2004; 125:83S-85S)

Key words: apoptosis; lung cancer; mutation spectrum; tobacco smoke

Abbreviations: CYP = cytochrome P450; ETS = environmental tobacco smoke; GSTM1 = glutathione-S-transferase M1; NO = nitric oxide

Physicians have long recognized that people differ in their susceptibility to disease. As early as the fifth century, Hippocrates wrote "Some men have constitutions that are like wooded mountains running with springs, others like those with poor soil and little water, still others like land rich in pastures and marshes, and yet others like the bare, dry earth of the plain." Today, we describe these observations as interindividual variation in disease risk manifested as gene-environment interactions, a notion embodying the concept that heritable traits modify the effects of environmental exposures. The heritable effects of genes in human cancer pathogenesis range from high penetrance with an attendant high likelihood of causing cancer to low-penetrant genes with an attendant increased risk of causing cancer, albeit less likely than that for high-penetrant genes. Nevertheless, the range from low-penetrant to high-penetrant genes is a continuum, and studies in animal models indicate that the effects of highly penetrant genes can be modified by other genes. In humans, high-penetrant genes that cause family cancer syndromes can have a substantial impact in the affected families (*eg*, Li-Fraumeni syndrome involving germline mutations in the p53 tumor suppressor pathway), but they affect only a small percentage of the population. In contrast, the manifestations of cancer susceptibility genes with less penetrance contribute to common sporadic cancers and, thus, affect a large segment of the population.

TOBACCO SMOKE AND LUNG CANCER RISK

The molecular epidemiology of lung cancer has received widespread attention because the primary etiology is well-established, namely, tobacco smoking, but it is also known that only some smokers develop lung cancer while others do not. Numerous studies have indicated that one reason for this is related to the presence of low-penetrant genes. Among the best examples of increased lung cancer risk (due to inherited susceptibilities and tobacco smoking) are studies from Japan,¹ in which a genetic role for the metabolism of carcinogens by cytochrome P450 (CYP) 1A1 and glutathione-S-transferase M1 (GSTM1) has been established. CYP1A1 activates and GSTM1 detoxifies the carcinogenic polycyclic aromatic hydrocarbons found in tobacco smoke. Furthermore, in the Japanese studies, the *CYP1A1* genetic variant that increases lung cancer risk has a greater effect in lighter smokers, where the risk of smoking approximately less than one pack per day for 40 years (odds ratio, 7) is similar to the risk of smoking > 40 pack-years and having the other *CYP1A1*

variant. We and others have proposed that genetic susceptibility will play a more prominent role in cancer cases with patients having low carcinogen exposure (*eg*, never-smokers exposed to environmental tobacco smoke [ETS] or long-time ex-smokers).¹ Our initial study² to test this hypothesis revealed that never-smoking women who develop lung cancer have a statistically significant increase in the GSTM1-null genotype. GSTM1 encodes an enzyme that detoxifies certain chemical carcinogens in tobacco smoke. A difficulty of such studies is in obtaining an accurate assessment of exposure to ETS. Improved analytic methods in the molecular dosimetry of ETS exposure also are needed (*eg*, the quantitative measurement of urinary metabolites of the tobacco-specific carcinogen NNK in women exposed to ETS).

MOLECULAR ARCHEOLOGY OF THE p53 MUTATION SPECTRA

The molecular archeology of the mutation spectra of tumor suppressor genes generates hypotheses concerning the etiology and molecular pathogenesis of human cancer.³ The spectrum of somatic mutations in the p53 gene implicates environmental carcinogens (*eg*, sunlight, aflatoxin B₁, and tobacco smoke), endogenous agents (*eg*, oxyradicals), and processes (*eg*, errors occurring during DNA repair and replication) in the etiology of human cancer.⁴ For example, we have observed that the increased production of nitric oxide (NO[•]) has a positive correlation with both an increased p53 mutation load in the colons of patients with ulcerative colitis⁵ and p53 C-to-T transitions in sporadic colon cancer cases, because of 5 MeC deamination during colon carcinogenesis.⁶ NO[•] also induces a DNA damage response pathway with an associated increased base excision DNA repair and p53 site-specific posttranslational modifications, both *in vitro* and in noncancerous colons from ulcerative colitis patients. These results implicate NO[•]-induced cellular stress in the molecular pathogenesis of cancer associated with chronic inflammation.

p53 TUMOR SUPPRESSOR PATHWAY

The p53 tumor suppressor pathway is frequently inactivated during the molecular carcinogenesis of human cancer.^{3,4} As described in other articles from this symposium, p53 inactivation leads to the diminished control of cell cycle checkpoints, decreased DNA repair, and increased genomic instability.⁷ For example, p53 up-regulates the Apaf1 gene,⁸ a member of the apoptosome, and it has been found to be critical for the protease activation of caspase-9.⁹ Our studies have focused on the mechanisms of p53-mediated apoptosis. Human p53-deficient cells have diminished nucleotide excision repair^{10,11} and base excision repair.^{12,13} Germline mutations in DNA helicases (*ie*, XPB, XPD, WRN, and BLM) lead to cancer predisposition, premature aging (WRN), and attenuated p53-mediated apoptosis.¹⁴⁻¹⁶ These and other results in-

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dicating that the p53-mediated apoptotic response to DNA damage involves both transcriptional and nontranscriptional mechanisms.

p53 was initially discovered to be a cellular protein bound to a simian virus-40 large T antigen, a viral oncoprotein with DNA helicase activity, in 1979. A decade later, p53 was identified as a tumor suppressor gene. p53 continues to be at the crossroads of molecular carcinogenesis and the molecular epidemiology of human cancer.

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Development of Lung Tumors in Mutant p53-Expressing Mice After Inhalation Exposure to Asbestos*

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(*CHEST* 2004; 125:85S–86S)

Key words: asbestos; lung cancer; p53

Abbreviation: SPC = surfactant protein C

The gene encoding the p53 tumor suppressor protein is commonly mutated in many human cancers, including lung cancer,¹ but p53 mutations are relatively rare in murine lung tumors induced by carcinogen exposures.² To model the pathogenesis of human lung cancers in mice, we disrupted wild-type p53 activities by transgenically expressing a dominant-negative form of p53 specifically in the lung epithelium using the human surfactant protein C (SPC) promoter, SPC-DNp53 mice.³ Distinct responses to fibrogenic agents have indicated that the transgene has altered the phenotype of SPC-DNp53 mice.⁴ However, the low incidence and delayed onset of lung tumor development in unexposed SPC-DNp53 mice imply that the oncogenic p53 transgene requires additional activities to complete the process of neoplastic conversion. Inhaled asbestos activates p53 expression at the sites of fiber deposition,⁵ and epidemiologic evidence indicates that exposure to asbestos increases the risk of lung cancer about fivefold.⁶ We postulate that the p53-mediated response to asbestos protects against the development of lung tumors. In accord with this postulate, a single exposure to an aerosol of asbestos for 5 h produced a significantly higher incidence of lung tumors in SPC-DNp53 transgenic mice than in simultaneously exposed nontransgenic littermates. These data indicate that compromised p53 function in the lung epithelium cooperates with asbestos in lung tumorigenesis.

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