The Molecular Epidemiology of Oxidative Damage to DNA and Cancer

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Oxygen is required for respiration and the energetic processes that enable aerobic life. A cost associated with oxygen use is free-radical formation, which damages genome stability and contributes to various processes including aging, degenerative diseases, and cancer (1,2). Foods including fruits, vegetables, tea components, and trans-fats; nutrients including vitamins C and E, selenium, beta-carotene, and dietary fish oil; chemotherapeutic drugs; radiation; infection; environmental exposures including air pollution; and hereditary and acquired conditions broadly contribute to or oppose free-radical formation and genomic damage (2–9). Individually and cooperatively, the action of modulators of oxidative DNA damage is the focus of intense study and controversy (10). Understanding the regulation of free-radical formation and its consequences may provide new insight into the etiology of cancer and lead to the development of effective chemoprevention agents.

Lung cancer is a logical disease for evaluating oxidative damage and the role of free radicals because the etiologic agents for lung cancer are tobacco carcinogens that are known to damage DNA (11). To understand the role of DNA repair activity in lung cancer, accurate, reproducible, and specific phenotype assays need to be developed and tested in human populations in molecular epidemiology studies. Results from such studies have shown that subjects with reduced DNA-repair activity, as measured by a variety of assays, have an increased risk of lung cancer. A variety of analytical techniques to assess oxidative DNA damage exist and have been recently reviewed (12). In this issue of the Journal, Paz-Elizur et al. (13) describe a DNA repair assay for the oxidative lesion 8-oxoguanine. The authors find that the 8-oxoguanine DNA N-glycosylase (OGG) activity is reduced in subjects with operable lung cancer. Here, I comment on the implications of these findings within the context of molecular epidemiology study designs.

The general challenges and pitfalls of molecular epidemiology studies including critical validation steps have been comprehensively reviewed (14). For evaluating the oxidative repair phenotype, i.e., a presumably stable host ability to repair a specific type of oxidative DNA damage known to result from mutagenic insults including tobacco smoking, there are five general categories of questions that can be addressed in a molecular epidemiology study (Fig. 1).

The first category is the relation between the oxidative repair phenotype and any of a broad range of epidemiologic exposures. This is fundamental not only because understanding the relation of the assay to basic human differences (i.e., age and sex) contributes to validation, but also because a putative relation between the DNA repair assay and lung cancer must be distinguishable from an effect of exposures associated with lung cancer on the assay itself. In lung cancer, it is important to establish at the outset whether the oxidative repair phenotype is associated with smoking, because the numerous chemicals in tobacco smoke include carcinogens that could deplete antioxidants or induce other alterations such as oxidative DNA base modifications (15). If the assay results are confounded by smok-
ing, then the phenotype will exhibit an apparent association with lung cancer but may not contribute at all to lung cancer susceptibility. Paz-Elizur et al. (13) found that OGG activity levels were similar between smokers and nonsmokers. It will be important to extend their findings involving tobacco, including whether there is a difference among never, former, and current smokers and between light and heavy current smokers. Many smokers who are the case patients in case–control designs will have actually stopped smoking some days or weeks before diagnosis because of respiratory symptoms directly or indirectly related to the diagnosis. It will also be important to establish whether other potential confounders such as nutrients and diet, environmental and occupational exposures, medication use, and comorbidities affect the OGG DNA repair assay. Understanding the possible influence of exposures on the phenotype will be relevant to establishing precisely how the process of oxidative DNA repair affects cancer.

The second category in molecular epidemiology study design is the relation between the oxidative repair phenotype and the disease, i.e., lung cancer. Typical questions involve establishing whether oxidative repair activity varies among lung cancer histologies or by other features of disease, such as stage and grade, and whether case–control differences may exist in other tobacco-related tumors. In this context, it is critical to determine whether the presence of the disease within the host affects the repair assay, because it is only practical to conduct complex phenotype studies in case–control settings where the assay is performed on material collected after the clinical diagnosis. DNA repair assays are often labor intensive (and therefore costly) and reproducibility issues arise when conducted on stored biologic specimens. Consequently, studying participants in cohort studies (where tissue and blood samples are stored before diagnosis) has not been feasible because it would require performing assays on prohibitively large numbers of potential control subjects over a long time period, while accumulating sufficient case patients. Case–control study findings can be biased if processes associated with lung cancer in the host such as cachexia, changes in diet, concurrent conditions (e.g., post-obstructive pneumonia), treatment, surgery, stress, and medication influence the DNA repair assay. Paz-Elizur et al. (13) provided some reassurance by showing that there was no correlation between OGG activity levels and the time between surgery and collection of the blood sample. Unfortunately, the high rate of lung cancer recurrence, even in surgically treated subjects, the lack of detailed clinical information, and the modest numbers studied leave room for questions. Stronger evidence could be provided by studying a selected group of early-stage case patients before and after surgery and establishing whether there is a change over time (16). Although Paz-Elizur et al. (13) showed no apparent difference in OGG activity levels between 17 patients with squamous cell carcinomas and 27 patients with adenocarcinomas, evaluating larger numbers of subjects with careful adjustment for tobacco use, sex, and age will be necessary to refine the precise relationship of OGG activity to these critical subcategories of lung cancer. It will be important to eventually conduct these studies in prospective settings where phenotypes can be measured before cancer is present (17,18). Toward this goal, Paz-Elizur et al. (13) also demonstrated that OGG activity from peripheral blood mononuclear cells stored for over a year remained stable.

The third category in molecular epidemiology study design involves the relation between the repair assay and genotype. Polymorphic variants of DNA repair genes are prime candidates in complex disease etiology because it is reasoned that genetically defective repair will amplify cancer risk due to cumulative carcinogenic exposures.

Studies of a variety of DNA repair genes have established that there are multiple variants involving different amino acid substitutions that are likely to affect DNA repair (19,20). An important implication of this body of work is that studies looking at only one or two polymorphisms in candidate genes such as XPD, XRCC1, and OGG1 and their relation to cancer will neither adequately capture the range of phenotypic variation nor reliably identify associations between the phenotype and cancer. It is not surprising, therefore, that a number of studies examining single or small groups of single-nucleotide polymorphisms, including OGG1—the gene for the DNA glycosylase that excises the oxidatively damaged form of guanine (8-hydroxyguanine or 7,8-dihydro-8-oxoguanine) (21)—in relation to lung cancer have yielded null or mixed results (22–24,25,26). The study by Paz-Elizur et al. (13) suggests that the relationship between OGG activity and OGG1 polymorphisms is one worthy of exploration. In the wider context, more comprehensive genotyping (19,20) will be required to sort out the relationships of complex diseases such as cancer to DNA repair and other gene families (27).

A fourth category in molecular epidemiology study design involves the relation between the oxidative repair phenotype and other biomarkers derived from lung cancer tissue or associated with lung cancer (28–31). Many related assays have associations with human cancers including the comet assay (28,29), and the bleomycin/benzo(a)pyrene diol epoxide repair (30,31) assay. Understanding their interrelations may help dissect the precise pathways that are most relevant to cancer and allow delineation of common or critical processes (32). More broadly, associating oxidative repair and other DNA repair phenotypes with other tumor markers such as somatic mutations in oncogenes or cyto genetic abnormalities (i.e., 3p deletions in lung cancer) will help refine our mechanistic understanding (33). Insights into oxidative repair may also emerge by studying its relation to biomarkers from the newer emerging areas of proteomics, angiogenesis, gene expression, epigenetic changes, and genomics within the context of molecular epidemiology studies (34).

The fifth category in molecular epidemiology study design involves establishing whether the oxidative repair phenotype is associated with disease outcome. This relationship can be most directly addressed in studies that include survival and treatment data, but indirectly by relating markers to tumor stage and grade, clinical data, and prognosis. Because radiation and chemo-

![Fig. 1. Categories in the molecular epidemiology of oxidative repair.](Image)
therapy influence oxidative processes and DNA repair, accounting for treatment effects in the study design is a requirement.

Integrated large studies will serve as the best platforms to address relationships from as many of the five areas as possible and thereby help to establish the role of oxidative repair phenotypes in human disease and cancer. Regardless of how refined a picture emerges from human and animal models, large-scale work in human populations will be required to confirm effects in realistic settings and to gauge public health implications. The investment should be worth it. Because DNA repair is implicated in processes that promote human cancer (35–37), the answers should yield ample dividends across the spectrum of cancer etiology, prevention, and therapy.

REFERENCES

(19) Mohrenweiser HW, Xi T, Jones IM. Many common amino acid substitution variants identified in DNA repair genes during population based screenings are predicted to impact protein function (abstract S826). Proc AACR 2003;44: p. 1166.