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-
lack of detailed clinical information, and the modest numbers
lung cancer recurrence, even in surgically treated subjects, the
between OGG activity levels and the time between surgery and
provided some reassurance by showing that there was no correlation
obstructive pneumonia), treatment, surgery, stress, and medica-
cachexia, changes in diet, concurrent conditions (e.g., post-
sufficent case patients. Case–control study findings can be bi-
control subjects over a long time period, while accumulating
performing assays on prohibitively large numbers of potential
DNA repair assays are often labor intensive (and therefore
diagnosis because of respiratory symptoms directly or indirectly
remained stable. Paz-Elizur et al. (13) also demonstrated that OGG activity
lung cancer but may not contribute at all to lung cancer suscep-
tivity. Paz-Elizur et al. (13) found that OGG activity levels
were similar between smokers and nonsmokers. It will be im-
portant 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 di-
agnosis 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
relevent 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 his-
tologies or by other features of disease, such as stage and grade,
and whether case–control differences may exist in other to-
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 bi-
ased 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 medica-
tion influence the DNA repair assay. Paz-Elizur et al. (13) pro-
vided 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 pa-
tients 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 pa-
tients with squamous cell carcinomas and 27 patients with ad-
enocarcinomas, evaluating larger numbers of subjects with care-
ful 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 geneti-
cally 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 look-
ing 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, in-
cluding 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 ex-
ploration. In the wider context, more comprehensive genotyping
(19,20) will be required to sort out the relationships of com-
plex 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/berzo(a)pyrene diole 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 cy-
togenetic 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, an-
giogenesis, 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.
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.