Mutagen Sensitivity: A Genetic Predisposition Factor for Cancer

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Abstract
Mutagen sensitivity, measured by quantifying the chromatid breaks induced by mutagens in short-term cultures of peripheral blood lymphocytes, has been used as an indirect measure of DNA repair capacity. Numerous epidemiologic studies have suggested that mutagen sensitivity is a cancer susceptibility factor for a variety of epithelial cancers. A recent classic twin study examined systematically the role of genetic and environmental factors on the mutagen sensitivity phenotype and provided compelling evidence that mutagen sensitivity is highly heritable. A new prospective analysis provides further support to the notion that mutagen sensitivity increases the risk of cancer. In this review, we briefly summarize nearly two decades of epidemiologic and genetic studies linking mutagen sensitivity and cancer risk. The evidence is becoming increasingly convincing that mutagen sensitivity is a risk factor for cancer development. [Cancer Res 2007;67(8):3493–5]

Introduction
It is widely recognized that both genetic and environmental factors play a significant role in cancer initiation. Aberrations in many cellular functions are involved in the etiology of cancer, among which DNA repair is of fundamental importance in maintaining genomic integrity and protecting against cancer. There is considerable interindividual variation in DNA repair capacity. Mutagen sensitivity, measured by quantifying the chromatid breaks induced by mutagens in short-term cultures of peripheral blood lymphocytes (PBL), has been used as an indirect measure of DNA repair capacity (1). The theoretical basis for mutagen sensitivity assay is that, in response to mutagen exposure, higher levels of genetic damage accumulate in people with suboptimal DNA repair capacity than in normal individuals. Therefore, the level of chromatid breaks induced by a mutagen challenge would reflect an individual’s ability to repair DNA damage. This hypothesis is supported by the observations that mutagen-induced chromatid breaks are highest in DNA repair deficiency syndromes, such as Fanconi’s anemia, ataxia telangiectasia, and xeroderma pigmentosum. The original mutagen sensitivity assay was developed using bleomycin as the mutagen challenge (1). The assay has since been expanded to use other etiologically relevant mutagens to assess genetic susceptibility to different cancers, such as benzo[a]pyrene diol epoxide, a tobacco polycyclic aromatic hydrocarbon, for smoking-related cancers (2); γ-radiation for breast cancer (3); and UV light for skin cancer (4).

In this review, we summarize nearly two decades of epidemiologic and genetic studies of mutagen sensitivity and cancer risk, highlighting evidences of genetic heritability.

Epidemiologic Evidence
Retrospective studies. Almost 100 epidemiologic studies have been published evaluating the association between mutagen sensitivity and cancer risk. There has been at least one positive study for most major cancers, including cancers of lung, breast, prostate, bladder, colorectum, brain, skin, head and neck, and soft tissue. Mutagen sensitivity has also been linked to the risk of developing premalignant lesions (5). The odds ratios (OR) from larger studies were generally in the range of 1.5 to 2.5, whereas smaller studies tended to yield higher ORs. The largest epidemiologic study of mutagen sensitivity with nearly 1,000 lung cancer cases and controls produced ORs of 1.63 [95% confidence interval (95% CI), 1.36–1.97] for bleomycin sensitivity and 1.85 (95% CI, 1.42–2.42) for benzo[a]pyrene dial epoxide sensitivity (6). Several studies suggested that combining mutagen sensitivity with other risk factors increases predictive power (2, 7). For example, joint effects between mutagen sensitivity, tobacco smoking, and alcohol consumption were observed in a multicenter case-control analysis of head and neck cancer (7). High bleomycin sensitivity and heavy tobacco smoking alone conferred 2.6-fold and 11.5-fold increased risk of head and neck cancer, respectively. However, the risk increased dramatically to 44.5-fold (95% CI, 17.4–114.0) in mutagen-sensitive heavy smokers. The consumption of alcohol further increased the risk to 57.5-fold (95% CI, 17.5–188.0). These high ORs underscore the importance of mutagen sensitivity as a cancer susceptibility marker, particularly when combined with other risk factors.

Prospective studies. There have been a few prospective studies of mutagen sensitivity scored before the onset of secondary tumors in head and neck cancer patients. The most recent study with 981 early-stage head and neck cancer patients reported that bleomycin sensitivity was associated with a significantly increased risk of developing secondary primary tumor and recurrence [hazard ratio (HR), 1.38; 95% CI, 1.02–1.86; ref. 8]. Chao et al. (9) recently reported the first prospective study before the onset of primary cancer. They measured baseline bleomycin sensitivity in PBLs from a cohort of 220 patients with Barrett’s esophagus, a precursor lesion of esophageal adenocarcinoma. They found that higher bleomycin sensitivity was associated with a significantly greater risk of developing aneuploidy, an intermediate end point biomarker in esophageal adenocarcinoma (HR, 3.71; 95% CI, 1.44–9.53), and a nonsignificantly greater risk of esophageal adenocarcinoma (n = 27; HR, 1.63; 95% CI, 0.71–3.75). Among patients with loss of heterozygosity at the p53 locus, increasing bleomycin sensitivity was associated with increased risk of both aneuploidy (P_trend = 0.005) and cancer (P_trend < 0.001). This prospective study provides strong support for the usefulness of mutagen sensitivity as a risk predictor for cancer development, particularly when combined with other events, such as loss of tumor suppressor (e.g., p53) function.
Genetic Heritability

Because most of the reports supporting mutagen sensitivity as a cancer susceptibility factor have been case-control studies that compared mutagen sensitivity among unrelated individuals, the merit of mutagen sensitivity, an intermediate phenotype, as a cancer risk factor has often been questioned for the possibility of "reverse causation." It is imperative to establish mutagen sensitivity as a heritable trait before it can be recognized as a genetic susceptibility factor for cancer. Evidence for the genetic heritability of mutagen sensitivity has been accumulating. A number of case-control studies showed that mutagen sensitivity remained a significant predictor of cancer risk independent of demographic and environmental risk factors (7, 10), although a few studies found the opposite (11). Family studies observed that first-degree relatives of mutagen-sensitive individuals were also mutagen sensitive, indicating genetic predisposition to mutagen sensitivity (3). Roberts et al. (3) studied the heritability of radiation sensitivity in PBLs in families of patients with breast cancer. They found that 62% of the first-degree relatives of radiation-sensitive patients were also sensitive compared with 7% of the first-degree relatives of patients with normal sensitivity. Segregation analysis of 95 family members showed clear evidence of high heritability of radiation sensitivity, pointing to a single major gene accounting for 82% of the variance between family members (3). Adema et al. (12) compared the numbers of chromatid breaks per cell in several different lymphoblastoid cell lines on radiation or bleomycin challenge and found a very strong correlation \( r = 0.99, P < 0.001 \) between the chromatid breaks induced by these two mutagens. The strong correlation suggests that radiation and bleomycin sensitivity may have similar underlying mechanisms. The most powerful method to assess the relative contribution of genes and environments to complex traits is the classic twin study design. The twin model, comparing similarities between monozygotic twins (genetically identical) and dizygotic twins (who share half their genes), is an ideal model to partition interindividual variability into components reflecting genetic and environmental factors. In a pioneering classic twin study involving 25 pairs of monozygotic twins and 14 pairs of dizygotes (twin pairs and siblings), Cloos et al. (13) estimated a high heritability of 75% for bleomycin sensitivity. A second twin study of 9 monozygotic twins and 10 dizygotic twins by Tedeschi et al. (14) also reported higher correlations of bleomycin sensitivity score in monozygotic twins as compared with dizygotic twins and yielded a heritability of 38%. In the third twin study with a substantially larger sample size (148 pairs of monozygotic twins and 82 pairs of dizygotes), the genetic heritability of mutagen sensitivity was evaluated using a biometric genetic modeling (15). In addition to bleomycin, other commonly used and biologically relevant mutagen challenges (benzo[a]pyrene diol epoxide, 4-nitroquinoline 1-oxide, and \( \gamma \)-radiation) were also evaluated. A pedigree-based maximum likelihood method \( (15, 16) \) was used to quantify the influences of genetic and environmental contributions to the trait. The null hypothesis that there is no genetic contribution to mutagen sensitivity was rejected for all the four mutagen-sensitive traits because the restricted model (assuming no genetic influence) fitted the data significantly worse than the full model (including all covariates and variance components), indicating that the genetic component contributed significantly to all four markers. The model generated a heritability estimate of 40.7%, 48.0%, 62.5%, and 58.8% for bleomycin, benzo[a]pyrene diol epoxide, \( \gamma \)-radiation, and 4-nitroquinoline 1-oxide sensitivity, respectively (Table 1).

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It is apparent that whereas heritability varies different agents, it plays the most prominent role in determining mutagen sensitivity for any mutagen evaluated. The nonshared and shared environment also contributes to mutagen sensitivity but to a lesser degree (Table 1). \( \gamma \)-Radiation seems to have the highest heritability (62.5%) and the least contribution from shared environment (0%) among these four mutagens, consistent with the high heritability of radiation sensitivity in PBLs observed by the aforementioned breast cancer family study (3). Bleomycin sensitivity has lower heritability in this twin study (40.7%). Cloos et al. (17) recently carried out microarray analysis in lymphoblastoid cells from bleomycin-sensitive and bleomycin-insensitive individuals to search for genes and pathways involved in bleomycin sensitivity. The profile of altered gene expression after \textit{in vitro} bleomycin exposure involved genes in many biological pathways, including cell growth and proliferation, cell cycle regulation, and DNA repair, but no specific pathway could be singled out to explain the difference in bleomycin sensitivity. These results suggest that there may not be a single major gene or a limited number of major genes determining bleomycin sensitivity. More likely, a group of low-penetration genes may determine bleomycin sensitivity.

### Table 1. Contribution of genetic and environmental variance components to mutagen sensitivity

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>Genetic contribution (%)</th>
<th>Nonshared environment (%)</th>
<th>Shared environment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleomycin</td>
<td>40.7</td>
<td>33.3</td>
<td>26.0</td>
</tr>
<tr>
<td>BPDE</td>
<td>48.0</td>
<td>31.8</td>
<td>20.2</td>
</tr>
<tr>
<td>( \gamma )-Radiation</td>
<td>62.5</td>
<td>37.5</td>
<td>0</td>
</tr>
<tr>
<td>4NOQO</td>
<td>58.8</td>
<td>39.7</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Abbreviations: BPDE, benzo[a]pyrene diol epoxide; 4NOQO, 4-nitroquinoline 1-oxide.
Perspectives and Conclusions

Over the past nearly two decades, mutagen sensitivity has been a commonly used phenotypic assay in cancer epidemiology. The epidemiologic evidence supporting its association with cancer risk is strong and consistent. More importantly, it is the only DNA repair assay whose heritability has been estimated by classic twin studies. Therefore, mutagen sensitivity is one of the best-established phenotypic susceptibility markers for cancer risk.

There remain some questions and issues to be addressed. The use of PBLs is only a surrogate for the target organ, and the correlation between surrogate and target tissue measurements has not been established. Epidemiologic analyses on the modulation of mutagen sensitivity by environmental factors (e.g., smoking, alcohol, and diet) have been inconsistent. The twin study showed that nonshared environmental factors exhibit considerable effects on mutagen sensitivity. Further studies are warranted to evaluate what environmental factors and to what degree such factors affect the mutagen sensitivity phenotype. The more interesting and important question awaiting study is to search for genes that are responsible for the mutagen sensitivity trait. There have been some reports of individual genotypes and genotype combinations in DNA repair genes and cell cycle checkpoint genes modulating the mutagen sensitivity phenotype. However, common polymorphisms in DNA repair genes that have been widely studied probably explain only a small amount of the variability in DNA repair capacity. The real genes that are responsible for the sensitivity to each mutagen are still elusive. The identification of such genes will not only improve our understanding of the mechanism of mutagen sensitivity phenotype and the origin of heritability but also provide novel candidate genetic markers for cancer risk assessment, clinical prevention, and treatment.

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References

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