The Smoking Gun and the Damage Done: Genetic Alterations in the Lungs of Smokers

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Considerable research has shown that tobacco use, particularly cigarette smoking, is the major cause of lung and other cancers (1). No other environmental, dietary, or lifestyle factor has been shown to have such an incontrovertible and important role in the development of cancer. Unfortunately, despite the enormous risks, a large fraction of our society continues to use tobacco, and many teenagers and young adults begin smoking each year. Smoking cessation and prevention strategies are being developed and tested, but their effectiveness has not yet been well established.

Given these sobering considerations, it is somewhat more encouraging to reflect on the remarkable progress that has been achieved over the past two decades in understanding what cancer is and how it develops. A widely accepted view is that cancers arise through a multistep process driven by acquired mutations and the clonal selection of variant progeny cells with increasingly aggressive growth properties. Moreover, as many in the cancer research community are well aware, considerable efforts are being expended to identify the specific constellation of mutations in particular types of cancer and to characterize the means by which the genetic alterations, either individually or collectively, subvert normal cell behavior and produce the insidious and destructive properties of advanced cancer cells. As a result of the molecular genetic studies, we now have powerful insights into the pathogenesis of cancer. However, the ultimate goals of the research efforts—effective treatment and prevention of cancer—remain essentially an implied future promise rather than a present reality. Hence, a jaded observer of the molecular genetic onslaught on the “cancer problem” might be prone to dismiss the findings of Mao et al. (2) reported in this issue of the Journal without reading beyond the title of the report. This would indeed be unfortunate. The studies of Mao et al. (2) have yielded new insights into our understanding of the genetic events in premalignant lung epithelium. Perhaps more noteworthy, their findings imply that the identification of clonal genetic alterations in asymptomatic individuals may have considerable utility in early detection and prevention strategies for lung and other cancers.

The concept that cancers arise from precursor lesions via a multistep process was first clearly articulated by Foulds (3) in the late 1950s. Subsequently, in 1976, Nowell (4) provided convincing arguments that a series of acquired (i.e., somatic) genetic alterations were responsible for the development of preneoplastic lesions and their subsequent clonal evolution to invasive and metastatic cancer cells. Molecular genetic studies of some cancers, such as those of the colon and rectum where precursor lesions (adenomas) can be readily obtained, have provided strong support for Nowell’s proposal (5,6). In cancers arising at other organ sites, such as the lung and the breast, clear-cut precursor lesions have been considerably more difficult to identify and procure. As a result, molecular genetic descriptions of the stepwise development of these cancers have been slower to emerge.

Nonetheless, the development of cancers of all types is believed to result from the accumulation of mutations in three classes of cellular genes, namely proto-oncogenes, tumor-suppressor genes, and DNA-repair genes. Oncogenic variant alleles of proto-oncogenes arise via point mutation, chromosomal rearrangement, or gene amplification. In contrast to the activating mutations in oncogenes, tumor-suppressor genes and DNA-repair genes are inactivated in cancer cells. DNA-repair genes are most often inactivated by point mutations or small deletions. However, a more diverse array of alterations, including localized mutations or deletions of large chromosomal regions, inactivate tumor-suppressor genes. Typically, the deletions involve only one member of an individual chromosome pair present in normal cells. Thus, each such deletion is referred to as an allelic loss or a loss of heterozygosity (LOH). In accord with Knudson’s (7) two-hit hypothesis, chromosomal regions affected by LOH are inferred to harbor a tumor-suppressor gene that has been inactivated by the occurrence of a recessive mutation in the retained allele.

Molecular genetic analyses of lung cancer have been intensively pursued over the past 15 years. Although more detailed histopathologic classification schemes exist, lung cancers can be readily divided into small-cell and non-small-cell types, with small-cell lung carcinoma (SCLC) accounting for approximately 20%-25% of lung cancer cases and non-small-cell lung carcinoma (NSCLC) accounting for the remainder. Both types may, in fact, arise from the transformation of a common pluripotent lung epithelial stem cell. However, SCLC and NSCLC have different natural histories and clinical courses as well as overlapping, yet distinct, profiles of genetic defects.

With respect to the genetic profile of SCLC, LOH at chromosome 17p is present in upwards of 75% of cancers, LOH at chromosome 3p is seen in nearly 100% of cancers, and LOH at chromosome 9p is present in more than 80% of cancers (8,9). There is substantial evidence that the p53 (also known as TP53) tumor-suppressor gene is the major target of inactivation on chromosome 17p in SCLC (8,10), while the fragile histidine triad (FHIT) gene or others may be inactivated by LOH at chromosome 3p (11,12). The gene targeted for inactivation by LOH at chromosome 9p remains poorly understood in most SCLCs, but it does not appear to be the p16 (also known as CDKN2)

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tumor-suppressor gene (9). In fact, p16 gene mutations are infrequent in SCLC, presumably because the frequent retinoblastoma gene mutations promote altered cell growth through similar mechanisms as p16 inactivation (13). In NSCLC, LOH at chromosome 3p has been seen in roughly 60% of cancers, LOH at chromosome 17p and p53 mutation have been observed in about 50% of cancers, and LOH at chromosome 9p and p16 inactivation are suspected to play a role in more than 50% of cancers (8,10,13,14).

As indicated above, because of the absence of grossly visible premalignant lesions, only very limited analyses of the genetic alterations in premalignant lung epithelial tissues had been previously undertaken. In some earlier studies of patients whose lung cancer had a p53 mutation (15-17), the identical mutation was found in the noncancerous bronchial mucosa adjacent to the tumors. Recent studies (18-20) have indicated that LOH at chromosomes 3p and 9p can also be identified in nonmalignant lung tissues of some patients with lung cancer. Despite these interesting observations, prior to the study of Mao et al. (2), no comprehensive investigation had been undertaken to assess the prevalence and nature of clonal genetic alterations in the lung tissue of asymptomatic individuals.

Mao et al. (2) describe their analyses of the genetic alterations and histologic abnormalities present in biopsy specimens from 54 current and former smokers and nine nonsmokers. In each of the current and former smokers, random bronchoscopic biopsy specimens from six preselected sites were analyzed for squamous metaplasia and atypia. In addition, LOH at chromosomes 3p, 9p, and 17p was used as a surrogate marker of tumor-suppressor gene inactivation in the tissues. The results of their study are quite intriguing. Although some differences were seen when the specimens of current smokers were compared with those of former smokers, clonal genetic alterations were surprisingly common in the nonmalignant lung epithelial tissue of both groups. LOH at chromosome 3p and squamous metaplasia were seen more frequently in current than in former smokers. Current and former smokers displayed similar frequencies of LOH at chromosomes 9p and 17p, with about 50% of each group exhibiting LOH at chromosome 9p in one or more biopsy specimens and about 20% exhibiting LOH at chromosome 17p in one or more specimens. Genetic changes were seen in biopsy specimens with and without squamous metaplasia. Only LOH at chromosome 3p appeared to be associated with metaplastic changes, although, as the investigators note, the metaplastic cells themselves may not harbor the clonal genetic alterations. Metaplastic changes in the lung, however, appear to reflect the potential field effects of smoking, and LOH at chromosome 3p and metaplasia may both be sensitive markers for these effects.

Despite the thorough analyses and interesting findings of Mao et al., some caution is urged in assessing the biologic and clinical significance of the results at this time, for two reasons. First, clonal genetic alterations have been described in lesions with very reduced or no malignant potential, including nondysplastic aberrant crypt foci and hyperplastic polyps of the colon (21,22), pancreatic duct hyperplasias (23), and endometriosis (24). Second, Mao et al. (2) studied only a limited number of lung tissues from nonsmokers. In 11 informative tissue samples from five nonsmokers, LOH at chromosome 3p was identified in only one sample and no cases of LOH at chromosome 9p were seen. Given these limitations, the investigators are careful to note that clonal genetic alterations may also be present in the lung tissues of individuals who have never smoked. However, the increased prevalence of LOH at chromosome 3p in the biopsy specimens from current smokers, when compared with the specimens from former smokers, strongly supports the proposal that smoking is the causal factor in the acquisition of the genetic defects. Additional studies will be of considerable help in more precisely defining the prevalence of genetic alterations in the nonmalignant lung epithelium of nonsmokers as well as in the lung tissue of current and former smokers.

The studies of Mao et al. (2) have interesting implications for our understanding of the pathogenesis of lung cancer and its clinical management, only a few of which will be outlined here. The investigators’ findings indicate that LOH at chromosome 3p may be an early event in the genesis of many lung cancers and that LOH at chromosomes 9p and 17p is more likely to be associated with tumor progression. Clones with LOH at chromosome 3p were less frequently detected in the lung epithelium of former smokers than of current smokers, implying that this LOH alone may give cells only a limited growth advantage. Additional genetic changes (e.g., LOH at chromosome 9p and/or 17p) are acquired as the clone progresses and may even be required for its persistence. While the timing of LOH at chromosome 9p and inactivation of the p16 gene in the development of common epithelial cancers is not well-known, inactivation of the p53 gene appears to have a role in lung tumor progression comparable to its role in colorectal and several other epithelial cancers (6,10). In addition, similar to findings obtained in prior studies of benign colorectal adenomas (5,6), premalignant lung lesions often have acquired multiple genetic changes without progression to overt cancer. The accumulation of multiple mutations may, in fact, confer increased malignant potential upon a cell. However, sizable cohorts of current and former smokers, as well as nonsmokers, must be studied to ascertain the number and constellation of alterations in premalignant lung epithelia that are associated with an increased risk of cancer. Despite the daunting challenge of such studies, the findings are likely to have critical importance in formulating improved early detection and chemoprevention strategies. Given the magnitude of the lung cancer problem, can we afford not to capitalize on such opportunities?

References

Helicobacter pylori and the Cell Cycle

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In 1994, the International Agency for Research on Cancer (IARC) classified Helicobacter pylori infection as "carcinogenic to humans (group 1)." This conclusion was based on significant evidence in humans while recognizing that there was inadequate evidence in experimental animals (1). The strongest evidence in humans came from three independent historic cohort studies showing that serologic evidence of H. pylori infection (documented decades before) was statistically significantly associated with the risk of gastric carcinoma. However, laboratory data on mechanisms of carcinogenesis were lacking. Unlike some chemical and physical carcinogens evaluated by IARC, H. pylori may not be a "complete" carcinogen and is not expected to induce cancer in animals without the help of other etiologic factors. Suggestive evidence of interaction between biologic and chemical carcinogens is provided by the very high incidence of gastric carcinoma in ferrets infected with chemical carcinogens is provided by the very high incidence of gastric carcinoma in ferrets infected with MNNG, a potent mutagen and gastric carcinogen (2). This finding suggests that Helicobacter infection potentiates environmental carcinogens. The search for gastric carcinogens in humans has focused on both the luminal (3) and tissue (4) microenvironments.

Given the difficulties of inducing invasive cancer in experimental animals infected with Helicobacter species, attention has been directed toward intermediate biomarkers of the carcinogenic process. In this issue, Peek et al. (5) examined the effect of bacterial strain differences on two critical components of the cell cycle: cell replication and apoptosis (programmed cell death).

This report comes at an opportune time because presently there is much interest in exploring if the virulence of certain H. pylori strains determines the outcome of the infection. In other fields of scientific research, cell cycle alterations are being evaluated to determine their possible role in carcinogenesis, including the possible identification of checkpoints that drive the cycle, the control of which may open new avenues of treating and preventing cancer in humans (6).

The model of Helicobacter-induced carcinogenesis is especially appealing to researchers for several reasons. One of these reasons is illustrated by the fact that chronic, prolonged infection with H. pylori increases the risk of gastric cancer in some groups of individuals but not in others. In the first category are patients with multifocal atrophic gastritis, some of whom develop gastric ulcer. In the second category are patients who develop H. pylori-associated, predominantly antral nonatrophic gastritis and duodenal ulcer (7). Since both outcomes are a consequence of H. pylori infection, the study of their biologic differences may help to identify events that are, as well as those that are not, part of the carcinogenesis cascade. Bacterial strains may be different in the two contrasting outcomes, but at the present time there are no


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