Etiology of the Mutational Spectrum of ras Genes in Human Carcinomas

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Among the many human behaviors that are associated with increased risk of malignancy, the smoking of tobacco has the most incontrovertible epidemiologic evidence linking it to malignancies of the upper aerodigestive and urinary systems. Sustained by nicotine addiction, tobacco smokers inhale a complex mixture of more than 60 known or suspected carcinogens, including polyaromatic hydrocarbons (PAHs), nitrosamines, aromatic amines, and inorganic compounds, over a period of decades (1). In this regard, the etiology of lung cancer and other smoking-related malignancies is better understood than that of other common adult carcinomas, where the proximate carcinogens, if they exist, have not been clearly identified. However, relatively little is known about the relative importance of specific tobacco smoke constituents or the molecular mechanisms whereby they lead to cancer.

Genetic alterations in human malignancies include deletions, gene amplifications, chromosomal rearrangements, and intragenic point mutations. The genes most commonly affected by sporadically acquired point mutations in cancers are p53 and the genes of the ras gene family, H-ras, K-ras, and N-ras. In epithelial malignancies, more than 80% of ras gene mutations occur in K-ras, and more than 80% of K-ras mutations occur at codon 12, whereas mutations at codons 13 and 61 are less common (2). This predominance of mutations at codon 12 of the K-ras gene is evident in both smoking-related and non-smoking-related malignancies, such as colorectal and pancreatic carcinomas.

Why do we observe cancer-associated gene mutations predominantly in one of the nine transforming codons of the ras gene family? The pattern of point mutations in cancers is the result of the interaction of three factors: 1) generation of an altered DNA base or nucleotide (e.g., carcinogen–DNA adduct formation or direct physical damage), 2) DNA repair (e.g., removal of carcinogen–DNA adducts), and 3) the biologic consequences of induced mutations. An additional level of complexity arises because many tobacco smoke constituents and environmental carcinogens undergo metabolic activation, producing reactive intermediates that may bind to DNA or, alternatively, undergo metabolic detoxification.

In this issue of the Journal, Feng et al. (3) hypothesized that codon 12 of K-ras may be especially prone to mutagenesis because it is more susceptible to DNA adduct formation and has less efficient repair of adducts than other transforming ras codons. They reached this conclusion after determining the relative amount of DNA adduct formation in synthetic oligonucleotides exposed to BPDE by using stable isotope-labeling mass spectrometry analysis. Together, these results support prior observations that adduct formation is primarily a function of localized DNA structure resulting from primary sequence and base modifications (i.e., methylation) and the chemical reactivity of the carcinogen (5,6). Feng et al. also demonstrated similar results in normal human fibroblasts and with other bulky chemical carcinogens, suggesting that the susceptibility of position 1 of codon 12 of the K-ras gene to adduct formation is likely to be generalizable across tissue types and to other guanine-binding mutagens.

Interestingly, position 1 of codon 14 of K-ras was at least as susceptible to adduct formation as codon 12, but adducts formed at codon 14 of K-ras were not as persistent (3). In H-ras, adducts were observed primarily at codons 1 and 3, although these apparently did not persist in the DNA. The underlying mechanism mediating these differences in DNA adduct repair efficiency at different locations within and between ras genes is currently unclear and is important to understanding tobacco smoke-induced carcinogenesis. The primary DNA repair processes known to be involved in the resolution of carcinogen–DNA adducts in human cells include mismatch repair, base-excision repair, nucleotide-excision repair, and direct reversal of DNA damage. The biochemical components of these pathways have been delineated, with acquired and inherited variation in DNA repair capacity being hypothesized as an important factor in determining the risk of smoking-related cancers, including lung cancer (7).

The mechanism of carcinogen–DNA adduct repair was not directly investigated by Feng et al. (3). Even though they found that DNA adducts formed at codon 12 of the K-ras gene may not be repaired as quickly as similar adducts at codon 14, it is possible that an alternative process (e.g., induction of damage-inducible genes leading to a higher rate of apoptosis of cells with adducts at codon 14 than of cells with adducts at codon 12) may be responsible for this observation. In addition, repair rates at codons 13 and 61 of K-ras were not assessed. Therefore, although decreased repair of adducts at codon 12 of K-ras is a...
plausible hypothesis to explain the persistence of adducts at this site relative to those at codon 14, the data presented by Feng et al. do not allow firm conclusions about relative repair efficiencies.

Do the observations of increased adduct susceptibility and possible decreased repair explain the preference for mutations at codon 12 of the K-ras gene in human cancers or the subset of tobacco smoke-related malignancies? A closer look at the mutation spectrum of K-ras suggests that the explanation may be more complex. In colorectal cancers, which are not strongly associated with tobacco use, about 80% of the mutations at codon 12 of K-ras occur at position 2 (8), a position that was not a hotspot for DNA adduct formation in the study by Feng et al. (3). In lung cancer, about 50% of the mutations at codon 12 of K-ras occur in position 1 (2) and this increased proportion of position 1 mutations is largely due to an increase in G to T transversions, which are thought to result from BPDE and other tobacco smoke-related adducts at this site (2). This latter observation supports the hypothesis that tobacco smoke-related carcinogens, such as benzo(a)pyrene, may preferentially form adducts at codon 12 of K-ras in lung cancer. However, the large proportion of mutations at position 2 of codon 12 of K-ras cannot be explained from the observations of Feng et al. regarding adduct formation and repair, at least without invoking additional mechanisms. Specifically, one might postulate position 2-specific adduction or repair of position 1 adducts through mechanism(s) that results in mutation at position 2. In this regard, it should be emphasized that mutations can occur at sites other than the adducted site, such as through error-prone repair mechanisms.

Why else might human carcinomas have a preference for mutations at codon 12 of K-ras? There is increasing evidence that not all ras genes are equivalent, but rather that they have tissue and species differences in regard to their signaling pathways and their biologic effects (9). Similar differences might also occur with different activating mutations, resulting in a selective advantage for cells with mutations at codon 12 of K-ras in epithelial cells. Therefore, understanding the mechanism(s) of carcinogen–DNA adduct generation and the processes that mediate DNA repair of specific adducts is essential for cancer risk assessment, and ultimately, cancer prevention. Furthermore, understanding the biologic basis for frequent mutations at codon 12 of K-ras in carcinomas may identify subtle but clinically significant differences in biochemical signaling pathways that can potentially be therapeutically targeted.

REFERENCES