Smoking and lung cancer—a new role for an old toxicant?

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Denissenko *et al.* (1) reported in 1996 that benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BPDE), a metabolite of the widely studied polycyclic aromatic hydrocarbon (PAH) carcinogen benzo[*a*]pyrene (BaP), produced a characteristic pattern of DNA damage in the *p53* tumor suppressor gene of lung cells. This pattern was remarkably similar to that observed in the commonly mutated *p53* gene isolated from lung tumors in smokers. In view of the central role of the *p53* gene in carcinogenesis, the results of this widely quoted study, which were reproduced subsequently with related PAH metabolites and by other methods (2, 3), have been described as a “smoking gun,” confirming that PAHs were the major carcinogenic agents in cigarette smoke. But life is not so simple. Cigarette smoke contains >4,000 compounds and >60 established carcinogens (4). In addition to PAH, there are carcinogenic nitrosamines, aromatic amines, aldehydes, volatile organic compounds, oxidants, and metals. As discussed in refs. 5 and 6, some of these compounds or their metabolites, similar to BPDE and other PAH diol epoxides, cause G-to-T transversions in DNA, as commonly observed in the *p53* and *K-ras* genes in smoking-induced lung tumors. These other agents might also cause patterns of DNA damage in the *p53* gene similar to those seen with BPDE. Confirming this possibility, Feng *et al.* (7) report in a recent issue of PNAS that acrolein, a toxic compound occurring in cigarette smoke at levels up to 10,000 times greater than those of BaP, produces essentially the same spectrum of DNA damage in the *p53* gene of human lung cells as that caused by BPDE. These results are summarized in Fig. 1 and suggest that acrolein and related compounds might be more important in cigarette smoke-induced lung cancer than previously recognized. Feng *et al.* also show that, like BPDE, acrolein reacts preferentially at methylated CpG sites in the *p53* gene. Moreover, acrolein inhibits repair of BPDE–DNA adducts. Considering that cigarette smoking causes 30% of all cancer deaths in developed countries (4), more research is necessary on the possible role of acrolein and related aldehydes as potential causes of lung cancer in cigarette smokers.

The presence of BaP and other carcinogenic PAHs in cigarette smoke is firmly established. Current levels of BaP

Fig. 1. Summary of results reported by Feng *et al.* (7), showing that acrolein and the BaP metabolite BPDE produce similar spectra of DNA damage in the *p53* gene of human lung cells and that these spectra resemble the pattern of mutations observed in the *p53* gene from lung tumors in smokers.

are 1.5–15 ng per cigarette, whereas those of other established PAH carcinogens are collectively ≈3–50 ng per cigarette (8). Hoffmann *et al.* (9) performed extensive studies showing that subfractions of cigarette smoke condensate highly enriched in PAH were its major tumor initiators on mouse skin. BaP and other PAHs cause lung tumors in laboratory animals when administered by inhalation or instillation in the lung or trachea (5). This research can be viewed in context of other studies that clearly

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established PAHs as the major carcinogenic agents in coal tars and other related combustion products, known causes of cancer of the lung and skin in humans (10–12). The uptake of PAH by smokers is greater than in nonsmokers, as demonstrated by quantitation of urinary PAH metabolites (13). Cigarette smoking induces PAH metabolism in human lung via the Ah receptor, leading to the formation of BPDE–DNA adducts, which have been detected in some analyses of human lung DNA (5). Together with the results of the Denissenko *et al.* study (1), there is considerable evidence supporting the role of PAH as one group of causative agents for lung cancer in smokers, and a recent working group of the International Agency for

Research on Cancer evaluated BaP as “carcinogenic to humans” (14).

Levels of acrolein in cigarette smoke are 18–98 µg per cigarette (8). Acrolein is an intense irritant and displays a range of toxic effects, including cilia toxicity (15, 16). With the exception of one study in which bladder tumors were produced by an initiation–promotion protocol in rats treated with acrolein (by i.p. injection) followed by dietary uracil, no carcinogenic effects of acrolein have been reported, perhaps because of efficient scavenging by glutathione and related sulfhydryls (17). An International Agency for Research on Cancer working group concluded that there was inadequate evidence for its carcinogenicity in experimental animals (16). Acrolein is widely believed to be responsible for the bladder toxicity of the chemotherapeutic agent cyclophosphamide. Thus, in laboratory animals, the carcinogenicity of acrolein, if it exists at all, is clearly substantially less than that of PAH or tobacco-specific nitrosamines.

My laboratory first characterized acrolein–DNA adducts in 1984 (18), and subsequent studies demonstrated that one of these adducts causes G–T mutations in human DNA (19), consistent with the data reported by Feng *et al.* (7) and with the most common types of mutations in the *p53* gene from smokers. Chung and coworkers (20, 21) have

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identified acrolein and crotonaldehyde–DNA adducts in a variety of human tissues by using a specially designed ^{32}P -postlabeling technique and report that their levels are higher in oral tissue from smokers than nonsmokers. My laboratory has recently obtained mass spectrometric evidence for the closely related crotonaldehyde–DNA adducts in human lung (22). Further mass spectrometric analyses of DNA from smokers' lungs for acrolein–DNA adducts are clearly needed. Many studies have used a general ^{32}P -postlabeling technique to analyze DNA from the lungs of smokers. The results of these studies consistently demonstrate the presence of a “diagonal radioactive zone,” or DRZ, in this DNA from smokers, with adduct levels consistently higher than in non-smokers (23). The origin of the DNA adducts comprising the DRZ is unknown, and they are often referred to in the literature as “hydrophobic DNA

adducts” or even “aromatic–DNA adducts.” Gupta and colleagues (24) recently demonstrated that these adducts are not derived from PAH or aromatic amines and presented evidence that they could be formed from aldehydes such as formaldehyde, acetaldehyde, and crotonaldehyde. Collectively, these data provide intriguing leads pertinent to a possible role of acrolein and related aldehydes in smoking-induced lung cancer, despite its apparently low carcinogenicity in laboratory animals. As pointed out by Feng *et al.* (7), identification of compounds responsible for lung cancer in smokers is critical, because ultimately levels of these compounds can be regulated to decrease the probability of cancer development in smokers, of whom there are ≈ 45 million in the United States and 1.2 billion worldwide (4, 25).

One caveat to the study by Feng *et al.* (7) is the concentrations used: $2\ \mu\text{M}$ BPDE and $60\ \mu\text{M}$ acrolein. These are

clearly higher than would be encountered in the lungs of smokers, possibly by as much as 100-fold. We do not know whether these high concentrations might produce spurious effects. It is possible that there might be an undetected toxic reaction resulting in the release of a common substance unrelated to BPDE or acrolein that damages the *p53* gene in the manner reported, although the results obtained in strictly chemical systems would argue against that. Dose–response studies would be desirable if sensitive enough methods can be developed.

In summary, the results reported by Feng *et al.* (7) are potentially important. Although not diminishing the solid evidence that PAH and tobacco-specific nitrosamines are causes of lung cancer in smokers, the results clearly raise the possibility that acrolein and related aldehydes are significant contributors to this generally fatal disease.

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