Wu et al. (1) have based their studies on the assay of Hsu et al. (2) who developed the bleomycin sensitivity assay in which the number of chromatid breaks induced by in vitro exposure to bleomycin in short-term cultures of peripheral blood lymphocytes was assessed to estimate host susceptibility to neoplastic cell transformation. Wu et al. (1) investigated the susceptibility to bleomycin and benzo[a]pyrene diol epoxide of chromosomes of lymphocytes from subjects who were previously treated with surgery, radiotherapy, or both for stage I or II squamous cell carcinoma of the head and neck and from healthy subjects. They concluded that the people who were sensitive to both mutagens were at 19.2-fold increased risk of cancer of the upper aerodigestive tract. In my opinion, Hsu’s hypothesis is not applicable in all cases. Indeed, both the ataxia-telangiectasia (AT) heterozygotes and the homozygotes are cancer prone. Only about 10%–20% of homozygotes develop neoplasms, mostly lymphomas and leukemias. Retrospective studies have indicated a substantial increase in all cancers for AT heterozygotes. But chromosomes of homozygote individuals are very sensitive to mutagens, whereas those of the heterozygotes are not (3). Recent investigations have shown that bleomycin was much less effective in inducing DNA damage in leukocytes from children with thyroid cancer than those from healthy subjects (4). Therefore, the sensitivity of lymphocytes of subjects to bleomycin is not a good predictor of high risk of cancer in all cases.

The authors have used the term “clastogenic” to include both mutagenic and carcinogenic effects. But the term clastogenic means chromosome damaging.

Wu et al. (1) have noted that the frequencies of spontaneous chromatid breaks are extremely low in normal subjects and in case patients. But Hastak et al. (5) have shown that patients with precancerous oral lesions, e.g., oral submucous fibrosis, oral leukoplakia, and oral lichen planus, showed an increase in the
number of micronuclei in circulating lymphocytes. The authors also showed that benzo[a]pyrene can induce a substantially increased level of micronuclei in lymphocytes of patients compared with those in the healthy subjects. But can we assume from these findings that these patients are at high risk for lung cancer? Moreover, some investigators (6) suggest that an increased level of chromosomal aberrations in peripheral lymphocytes reflects an enhanced cancer risk (e.g., only the spontaneous breaks, but not bleomycin-induced breaks, in cells of workers exposed to different carcinogens or mutagens). Studies in Nordic and Italian cohorts have shown that the standardized incidence ratio for all cancers is elevated among subjects with a high frequency of chromosomal aberrations. These investigators conclude that the frequency of chromosomal aberrations in peripheral blood lymphocytes is a relevant biomarker for cancer risk in humans. So, there are two contradictory ideas for prediction of cancer susceptibility based on 1) mutagen-induced chromosomal breaks or 2) spontaneous chromosomal breaks. But, in my opinion, the conclusion would be more realistic if they are supported by the cancer incidence data.

In conclusion, the data presented by Wu et al. (1) concerning the calculation of risk of cancer of upper aerodigestive tract in subjects sensitive to mutagens are hypothetical; and only future prospective investigations will show whether conclusions of their paper have practical significance.

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REFERENCES


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RESPONSE

We thank Dr. Nersesyan for his interesting comments on our manuscript and take this opportunity to clarify aspects of our analysis.

We believe that Nersesyan has misinterpreted the data from the study by Tomanin et al. (reference 3 in the Nersesyan correspondence). Tomanin et al. reported that bleomycin-induced chromatid breaks were significantly higher in ataxia-telangiectasia (AT) patients than in control subjects, which is consistent with our findings. Nersesyan refers to this paper to support his statement that "chromosomes of homozygote individuals are very sensitive to mutagens, whereas those of the heterozygotes are not." Tomanin et al. used bleomycin in their assay to determine if there was "a possible increased sensitivity of heterozygotes to possible diffusible clastogenic factors present in the plasma of L-B (Louis-Bar) serum." This assay was not designed to test the sensitivity of heterozygotes to bleomycin itself nor did Tomanin et al. indicate that it was designed to do so.

Secondly, Nersesyan states that bleomycin was less effective in inducing DNA damage in leukocytes from children with thyroid cancer than those from healthy subjects. In previous studies, we have demonstrated that, although bleomycin sensitivity is consistently elevated in patients with cancers of the head and neck, colon, and lung, bleomycin sensitivity of patients with tumors of the breast or central nervous system did not differ from those shown by control individuals (1). Different carcinogens or mutagens act on cells through different molecular mechanisms, and they affect different repair pathways and show different sensitivities. We have found that a person who is sensitive to one mutagen can be resistant to others (2). The bleomycin sensitivity assay has also been expanded to measure the risk of other cancers by replacing the test mutagen, bleomycin, with 4-nitroquinoline-oxide (4-NQO), γ-radiation, and benzo[a]pyrene diol epoxide (BPDE). Our research group and others have shown that 4-NQO sensitivity can be used to predict melanoma risk (3), that γ-radiation sensitivity can be used to determine glioma risk (4), and that BPDE sensitivity can be used to estimate smoking-related cancer risk (5). Therefore, it is possible that the thyroid cancer patients are not sensitive to bleomycin. Furthermore, Nersesyan refers to a paper by Frenzilli et al. (reference 4 in the Nersesyan correspondence) that employs the Comet assay and not a mutagen-sensitivity assay. We do not know whether these two assays measure the same mechanisms of DNA repair. Further studies are needed to evaluate the relationship between these two mutagen-sensitivity measures.

Nersesyan seems to consider all micronuclei formation to be the result of chromosome breakage. Whereas some broken chromatid or isochromatid fragments can indeed form micronuclei, most micronuclei are caused by mitotic disturbances (6). Under the influence of a mitotic poison (e.g., vinca alkaloids, Taxol, etc.), mitotic cells fail to enter anaphase. The chromosomes enter the so-called c-anaphase and c-telophase states and eventually become micronuclei. Even when the mitosis-arresting substance is removed under experimental conditions, and the arrested metaphases can resume anaphase movement, lagging chromosomes can become micronuclei.

Nersesyan contrasts our findings with those of Hagmar et al. (reference 6 in the Nersesyan correspondence), who reported that chromosomal aberrations were a relevant biomarker of cancer risk. It must be stressed that that study was conducted on a cohort of workers occupationally exposed to a variety of potential mutagens. Hsu et al. (7) examined the spontaneous chromosome breakage frequencies in untreated lymphocytes in 182 randomly selected normal individuals and 232 cancer patients.
He found that the frequencies of spontaneous chromatid breakage were low (break per cell, range = 0.00–0.12). The average break per cell number was 0.0183 for normal subjects and was 0.0223 for cancer patients. Please also note that Hagmar et al. (reference 6 in the Nersesyan correspondence) evaluated chromosome aberrations, not chromatid breaks. Furthermore, they did not present mean spontaneous aberration values nor did they evaluate bleomycin-induced chromatid breaks in that paper.

We agree that further study of cohorts using mutagen-sensitivity measures would provide valuable insights.

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