Mutagen Sensitivity as a Susceptibility Marker for Human Hepatocellular Carcinoma

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Abstract

Although the pathogenesis of hepatocellular carcinoma (HCC) remains poorly understood, hepatitis B virus and dietary aflatoxin exposures are established etiological factors for this disease. We conducted a pilot study of 28 patients with HCC and 110 healthy controls matched for age, sex, and ethnicity to determine whether constitutional genetic instability, based on the quantification of mutagen-induced chromatid breaks in cultured lymphocytes, modifies an individual's risk of HCC development. The mean numbers of bleomycin-induced breaks per cell for cases and controls were 0.92 and 0.55, respectively (P < 0.0001). For benzo(a)pyrene diol epoxide (BPDE) sensitivity, the values were 0.90 for cases and 0.46 for controls (P < 0.0001). Nearly 68% of the cases but only 27% of the controls exhibited bleomycin sensitivity (i.e., had ≥0.68 breaks per cell). Eighty% of the case group but only 22% of the control group exhibited BPDE sensitivity (i.e., had ≥0.58 breaks per cell). On multivariate analyses, both bleomycin sensitivity and BPDE sensitivity were associated with significantly elevated risks for HCC, with odds ratios (95% confidence intervals) of 5.63 (2.30, 13.81) and 14.13 (3.52, 56.68), respectively. For individuals who were sensitive to both assays, the risk was 35.88. A synergistic interaction between the bleomycin sensitivity and BPDE sensitivity in HCC risk was suggested. These preliminary findings suggest that differences in host factors related to the predisposition to chromosome breakage, the capacity for DNA repair, or both may be involved in HCC development by influencing the predisposition of hepatitis B virus integration into human DNA or that the carcinogens induced DNA damage susceptibility. A larger study is needed to confirm these intriguing results.

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Introduction

Although chromosome breakage syndromes reflect extreme examples of the susceptibility syndrome, there may be, within the general population, individuals with latent instability that is unmasked by mutagen challenge in vitro. On the basis of this hypothesis, the mutagen sensitivity challenge assay has been used as a measure of constitutional genetic instability (net results of DNA repair capability and initial genetic instability; Refs. 1–4). Numerous studies have shown that mutagen sensitivity is an excellent independent risk predictor for developing cancer (5–8). However, no studies are available regarding the relationship of mutagen sensitivity and risk of HCC.

HCC is one of the most frequently occurring malignancies on a global scale, with an incidence of 20–150 cases per 100,000 people per year in some areas of Asia and Africa. It occurs much less frequently in Western Europe and the United States, with 1–5 cases per 100,000 people per year (9). HBV infection and dietary aflatoxin exposures are established etiological factors, and HCV infection, alcohol consumption, and several genetic metabolic disorders may also be independent etiological factors for HCC (10–12). Viruses, like chemical and physical carcinogens, are clastogenic, i.e., capable of causing chromosome breakage. It is reasonable to suppose that individuals who are sensitive to a clastogen because of higher initial sensitivity or inability to repair clastogen-caused DNA damage (including viral integration) may also have an increased risk of developing HCC.

In the pilot study reported here, we investigated whether mutagen sensitivity, as measured by an in vitro lymphocyte assay, as an index of constitutional genetic instability influenced the risk of HCC development. To do so, we determined how many chromatid breaks were induced by bleomycin, a radiomimetic agent, and by BPDE, a tobacco mutagen, in short-term cultured peripheral blood lymphocytes.

Materials and Methods

Study Subjects. Cases were patients with previously untreated histologically confirmed HCC identified from The University of Texas M. D. Anderson Cancer Center from 1996 through 1997. For convenience, in this pilot study, the control subjects were selected from an ongoing funded lung cancer case-control study, in which controls are being recruited from clinics of the largest health maintenance organization in the Houston metropolitan area. Using a frequency-matching approach, we selected previously accrued comparison subjects who were matched to the cases for age, sex, and ethnicity.

The epidemiological data were collected by personal interviews. The data included information on sociodemographic

1 The abbreviations used are: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; BPDE, benzo(a)pyrene diol epoxide; HBsAg, HBV surface antigen; OR, odds ratio.

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Characteristics and lifestyle habits. Ten ml of blood were drawn from patients and control subjects into heparinized tubes for HBV marker and cytogenetic analysis.

**Chromosome Analysis.** Bleomycin and BPDE were selected as test mutagens to induce chromatid breaks. Bleomycin is a radiomimetic agent that causes single- and double-stranded breaks requiring base excision repair; BPDE is the activated form of benzo(a)pyrene, a tobacco-specific agent, that forms bulky DNA adducts and is repaired by nucleotide excision. The bleomycin sensitivity assay has been well established (12). The BPDE dose and time point were selected based upon an experiment on 5 cell lines and 133 human peripheral blood lymphocyte samples. (+/-)-Anti-BPDE was purchased from Midwest Research Institute (Kansas City, MO). We used tetrahydrofuran (Sigma Chemical Co., St. Louis, MO) as the solvent. The 1 mm stock solution was aliquoted in microcentrifuge tubes (500 μl each) and stored at -20°C in the dark.

Standard lymphocyte cultures were established as described previously (13). On day 3 of incubation, the cultures were treated with 0.03 units/ml bleomycin for 5 h for the bleomycin sensitivity assay and 2 μM BPDE for 24 h for the BPDE sensitivity assay. During the last hour, the cells were treated with 0.04 μg/ml colcemid to arrest them in mitosis before they were harvested for conventional air-dried preparations. All prepared slides were coded and stained with Giemsa stain. Breaks were counted in 50 metaphases and expressed as the average number of breaks per cell. Only frank chromatid breaks or exchanges were recorded; chromatid gaps or attenuated regions were disregarded. The slides were read without knowledge of the subjects’ case-control status.

**HBV and HCV Testing.** HCV antibody (anti-HCV) was tested by a second-generation ELISA (Abbott Laboratories, North Chicago, IL), and positive results prompted confirmatory testing by radioimmunoblotting assay (Chiron). HBsAg was tested by ELISA (Abbott Laboratories, North Chicago, IL). A single specimen positive for anti-HCV or HBsAg was considered an adequate presentation of probable chronicity because most positive results by those tested remain so on follow-up testing, except those presenting with acute clinical hepatitis.

**Statistical Analysis.** To test for significant associations between mutagen sensitivity and cancer risk, univariate ORs were calculated as estimates of the relative risk. Ninety-five% confidence intervals were computed by the method of Woolf (14). Logistic regression was conducted with STATA statistical software (15). Mutagen sensitivity was treated as a continuous variable and was also categorized by the 75th percentile breaks/cell value (0.68 breaks/cell for bleomycin sensitivity and 0.58 breaks/cell for BPDE sensitivity), based on the distribution in the control subjects.

**Results**

**Distribution of Selected Demographic Variables.** There were 28 HCC cases and 110 frequency-matched healthy control subjects. There were 22 Caucasian-Americans, 3 Mexican-Americans, and 3 African-Americans in the case group and 88 Caucasian-Americans, 12 Mexican-Americans, and 10 African-Americans in the control group. Thirty-nine% of the patients in the case group were women, compared with 42.7% in the control group. The mean age for the cases was 58.32 years, compared with 60.65 years for the control subjects. There were no significant differences in age, sex, and ethnicity between case and control groups. In terms of family history of cancer, 36% of the case group had at least one first-degree relative with cancer, compared with 53.2% in the control group.
Table 1. On univariate analysis, both bleomycin and BPDE sensitivity were associated with significantly elevated risks for HCC, with ORs (95% confidence intervals) of 5.63 (2.30, 13.81) and 14.13 (3.52, 56.68), respectively.

Stratified analyses were performed to examine the interaction between bleomycin sensitivity and BPDE sensitivity (Table 2). Subjects who were not sensitive to either mutagen were used as a reference group. Seven of the (46.7%) cases and four of the (5.9%) controls exhibited sensitivity to both mutagens. Bleomycin sensitivity alone carried an OR of 1.71 (0.14, 20.51); BPDE sensitivity alone carried an OR of 9.32 (1.59, 54.69). Individuals who were sensitive to both mutagens had an OR of 35.88 (5.49, 234.43). This combined OR suggests that the joint effect for the two-mutagen sensitivity was greater than multiplicative (i.e., 35.88 > 1.71 \times 9.32).

There were no significant associations between mean breaks per cell and age, sex, pack-years, smoking status, or viral markers (data not shown). We measured HBsAg and anti-HCV in patients with HCC. Four of 6 patients who were anti-HCV positive were also mutagen sensitive. Because viral marker data were not available in our control samples, we were not able to evaluate the interaction between HBV/HCV infection and mutagen sensitivity in cancer risk.

Discussion

Data from this preliminary study suggest that sensitivity to bleomycin and BPDE is associated with significantly increased risk of HCC. Therefore, differences in host factors related to the predisposition to chromosome breakage, the capacity for DNA repair, or both may be involved in HCC development by influencing the predisposition of HBV integration into human DNA or susceptibility to carcinogens, such as aflatoxin. Assessing such differences between individuals may provide new insights in HCC research.

Different mutagens act on cells through different molecular mechanisms and so may activate different repair pathways. Bleomycin can cause single- and double-stranded breaks, and repair of bleomycin-induced DNA lesions primarily requires the action of polymerases. BPDE forms covalent adducts upon interaction with DNA in vitro or in vivo and requires a nucleotide excision repair system (17, 18). A key feature of nucleotide excision repair is the introduction of two incisions into the damaged DNA strand, one on each side of the DNA lesion. The size of the repair patch formed during nucleotide excision repair is ~20–25 nucleotides long. Then, DNA polymerase δ and ε are responsible for nucleotide excision repair synthesis. The single-stranded binding protein, HSSB, can also modulate the repair synthesis. Any defects that result in abnormal incision or repair synthesis may lead to elevate BPDE sensitivity. BPDE sensitivity and bleomycin sensitivity, therefore, probably represent different repair pathways or two different initial sensitivity pathways. We noted a synergistic interaction between BPDE sensitivity and bleomycin sensitivity in HCC risk, suggesting that, using these two assays in parallel, we might be able to refine our ability to define high-risk subjects.

There are other lines of evidence suggesting that genetic instability and DNA repair deficiency play roles in HCC. Collier and Bassendine (19) reported that deficiency of the repair enzyme for the highly promutagenic and potentially carcinogenic DNA base lesion, O6-methylguanine, underlies the increased risk of HCC seen in patients with cirrhosis. Lee et al. (20) reported that HBV X protein interacts with a cellular protein, designated XAP-1, which is the human homologue of the monkey UV light-damaged DNA-binding protein that functions in DNA repair. This interaction suggests that the cellular DNA repair process may be affected by HBV and that the resulting genetic instability may contribute to hepatocellular carcinogenesis. Alternatively, HBV integration may activate the XAP-1 repair pathway with XAP-1 deficiency, causing genetic instability, which may contribute to hepatocellular carcinogenesis. It is possible that BPDE DNA adducts and HBV integration may require similar DNA repair pathways. However, until the molecular basis of mutagen sensitivity is categorized further, the increased mutagen sensitivity in patients with HCC must be interpreted as a measure of relative susceptibility that may represent multiple processes.

One potential concern is that bleomycin and BPDE sensitivity may represent an effect rather than a cause of cancer. However, there were no significant associations between mean breaks per cell and age, sex, pack-years, smoking status, or viral markers. There was also no association between mean breaks per cell and family history of cancer in their first-degree relatives (data not shown).

In summary, this pilot study is the first to indicate that increased sensitivity to mutagens may be associated with risk of developing HCC. A considerably larger study would be required to fully explore the possibility of interactions among mutagen sensitivity, HBV and HCV infection, and epidemiological risk factors in HCC carcinogenesis. Further studies on the role of DNA repair deficiency in HCC risk are warranted.

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References


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