[CANCER RESEARCH 54, 5022–5023, September 15, 1994]

Letter to the Editor

Correspondence re: Y. Fujimoto *et al.*, Alterations of Tumor Suppressor Genes and Allelic Losses in Human Hepatocellular Carcinomas in China. Cancer Res., *54:* 281–285, 1994.

It appears that the main assumption for investigating the chief determinants of liver cancer in a case-control study in two areas of China, Beijing and Qidong, by Fujimoto *et al.* (1) was that, while HBV[1] exposure was assumed to be equivalent for these two regions, aflatoxin exposure, in contrast, was said to be "low" in Beijing and "high" in Qidong. Based on these assumptions and on their findings that hepatocellular carcinoma gene alterations were distinctly different for these two regions, the authors concluded "... that aflatoxin B_1 and/or other environmental carcinogens may contribute to this difference."

However, no evidence or references were provided for their dual assumptions of AF and HBV exposure levels for these two regions. I believe this to be a serious oversight, especially given the very small number of cases from Beijing for whom this generalization was applied. I believe that these assumptions are questionable for the following reasons.

First, we also recorded for the years of 1983 (2) and 1989[2] exposure to AF and HBV (surface antigenicity and antibody to the core protein) for Qidong County and a neighboring county near the Beijing-Tianjin urban corridor (Huanghua County). AF exposure was estimated by measuring urinary AF metabolites (Table 1) in the 1983 samples as total oxidized AF metabolites by a method used by Groopman *et al.* (Ref. 3; $r = 0.26$, $P = 0.10$, compared with AF B_1 intake) and in the 1989 samples as AF M_1 ($r = 0.55$, $P < 0.000001$, compared with AFB1 intake; Ref. 3) using a new, much more sensitive (100-fold) antibody affinity column/high performance liquid chromatography column/fluoresence detection combination. Although AF exposure was slightly lower for Beijing/Tianjin than for Qidong, these differences were not considered significant, especially when compared with the full range for all counties (2–7% of this range) and when comparing intakes of the chief food source of AF, corn. HBV exposure for 1983, recorded as the percentage prevalence of HBV-persistent carriers, was 3-fold higher for Qidong (16 *versus* 5% for both sexes, 100 individuals in each county).

Second, broad generalizations about urban-rural differences in AF exposure, especially for two sites only, is too uncritical. For example, in previous work in the Philippines in the late 1960s and early 1970s, we found that urban areas tended to have higher aflatoxin consumption than did rural areas, primarily because of the escalation of AF concentration in food products following processing (4, 5).

Third, AF exposure exhibits enormous variation between individuals and over time (considered in terms of months to years) because of the highly skewed distribution of AF residues in food (6–8 orders of magnitude in native fruit kernels, which may or may not be selected for consumption).

And finally, the observation in this study that there were "multiple alterations in DNA located on different chromosomes," along with the subsequent suggestion that these genomic changes "may be involved in the development of hepatocellular carcinoma," makes the authors' conclusion on aflatoxin all the more questionable. Indeed, it is puzzling why these authors emphasize the hypothesis that this disease is attributed to viral and chemical carcinogen factors, while ignoring the *in vitro* evidence that humans are unusually resistant to this carcinogen (6, 7) and/or to the human (2) and animal (8) evidence that nutrient intake very likely is the most relevant controlling factor in gene expression and disease occurrence, regardless of carcinogen or viral status.

Table 1 *AF exposure indicators*

Year (exposure)	Beijing-Tianjin[a]	Qidong	Full range
1983 (AF)[b]	69 (26)	114 (25)	0-611
1983 (corn, g/d)	171	164	0-660
1989[c]	5.5 (30), 2.8 (30)	2.2 (30), 0.0 (30)	0-108

[a] Number of male subjects in parentheses.
[b] Total AF metabolites, ng/4-h morning collection.
[c] AF M_1 (ng), first morning void; analysis of pooled urine samples, one assay per county in 1983 samples, duplicate assays per county in 1989 samples.

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Reply

Dr. Campbell (1) questions some of the "assumptions" and "conclusions" in our recent paper (2) in which we examined alterations of tumor suppressor genes and allelic losses in human hepatocellular carcinomas from studies conducted in the Qidong province and Beijing in China.

Dr. Campbell maintains that we have not adequately documented and/or referenced the difference in AFB1[1] exposure levels between Qidong and Beijing and the similarity of HBV exposure at these sites.

Received 2/3/94; accepted 7/20/94.
[1] The abbreviations used are: HBV, hepatitis B virus; AF, aflatoxin.
[2] Unpublished data.

Received 7/11/94; accepted 7/20/94.
[1] The abbreviations used are; AFB1, aflatoxin B_1; HBV, hepatitis B virus; LOH, loss of heterozygosity.

The primary reference demonstrating the difference in AFB1 exposure levels between Qidong and Beijing is that of Sun *et al.* (3), which is also referenced by Hsu *et al.* (Ref. 8 in our paper). It would have undoubtedly been better if we had directly referred to Sun *et al.* (3) in our paper rather than using the important paper of Hsu *et al.* as a general reference to cover both AFB1 exposure and the codon 249 mutation of *p53*. Interestingly, Dr. Campbell's own data shown in Table 1 indicate that the AFB1 exposure in Beijing/Tianjin is lower than that in Qidong, although he considers these differences (without statistical evaluation) insignificant. As to the difference in HBV exposure level in Beijing and Qidong, we simply stated in our paper that "exposure to HBV is high" in Beijing, but we did not claim that the HBV exposure was the same in both locations. The important point is that all the patients from Beijing were HBV positive. We believe, therefore, that our assumptions regarding AFB1 and HBV exposure are well founded and the concern of Dr. Campbell may not be valid.

Dr. Campbell rightly pointed out the small number of cases from Beijing. We did, of course, recognize this and indeed we pointed this out in the paper. Nevertheless, we believe our data are substantial enough to test the hypothesis proposed in our paper, *i.e.*, "that the causative agent(s) for the mutation of codon 249 in the *p53* gene may also contribute to loss of specific chromosomes or genes, resulting in a unique spectrum of LOH in tumors harboring the 249 mutation."

The data clearly showed no significant differences in LOH of marker loci between tumors in Qidong harboring the codon 249 mutation and those having other types of mutations or no detectable mutations in exons 5, 6, 7, and 8 of the *p53* gene. Thus, our results suggest that the agent(s) responsible for the mutation of codon 249 appear(s) not to increase the frequency of LOH at loci that are known to be associated with hepatocellular carcinoma. Also, our results show a distinct difference in the pattern of allelic losses between hepatocellular carcinomas in Qidong and Beijing. Since strong evidence now exists that the frequency of the codon 249 mutation parallels the level of AFB1 exposure (4, 5), we believe our suggestion that AFB1 and/or other environmental carcinogens may contribute to this difference is plausible.

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