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October 15, 2003

Dear Editor:

Thank you for the opportunity to revise our manuscript entitled “Tobacco, Alcohol, and p53 Overexpression in Early Colorectal Neoplasia” to BMC Cancer. We greatly appreciate the thoughtful review by the referees. The revised manuscript reflects the comments from the reviewers. Below is a summary of these changes.

Referee 1:

The authors should provide more details on the selection of patients: The methods section has been revised to clarify the selection of patients. The parent study was conducted within three New York colonoscopy screening clinics. Patients received colonoscopies for a variety of reasons including but not limited to symptoms as well as family history of colorectal cancer. Many of the colonoscopies revealed other pathologic conditions not of interest to the current study including hyperplastic polyps. 508 subjects had no polyps (either adenomatous or hyperplastic) and these subjects were classified as the control group. All of the subjects were asked to complete a questionnaire – 81.9 completed the questionnaire, which we believe is a very good response rate for epidemiologic studies. Moreover, we don’t believe that selection bias would influence the results of the study since the 18.1% who chose not to participate did not know their p53 protein overexpression status.

Referee 2:

Change chromagin to chromogen. The text was changed.

1. The definition of “carcinoma insitu” or intramucosal carcinoma should be abandoned, since they lead to confusion. We thank the reviewer for reading another paper by our group which recommends for epidemiologic analyses to include cases of carcinoma and intramucosal carcinoma together since it is difficult to reliably distinguish between the two. This is why for the main analyses we group these cases together and subdivided the case groups only by p53 status. However, to examine the overall prevalence of p53 protein overexpression, we decided to report the results separately for each case group as they to represent different steps along the adenoma-carcinoma continuum. For risk factor associations, however, we believe it is better to group these cases together as we have done in these analyses.

2. Was the lower prevalence for p53 protein overexpression a result of degradation due to the use of stored paraffin slides? As the reviewer correctly points out, some degradation is always possible when using stored paraffin slides. The time between cutting and staining, however, was very short and
ranged from 1 week to 1 month on average thus reducing the possibility of degradation. However, even if there was some degradation which will alter the absolute results, the relative results if anything would be stronger without this degradation. Thus, we can be confident that, if anything, our findings are underestimates of the true association. In the introduction, we state the reported ranges for protein overexpression along the adenoma-carcinoma sequence, the range is quite wide (7-60%) with some studies reporting smaller prevalences.

3. Please mention the positive control used. The text has been changed to reflect the fact that a positive and negative control was used for each batch.

4. The aim of the abstract and the paper seems to know how epidemiological risk factors relate to p53 status, instead the method section the aim of the analysis seems to know whether p53 status was different for risk factors for colorectal neoplasia relative to polyp free control group. The reviewer is correct to state that the aim of the overall paper is to examine how colorectal risk factors differ by p53 status in early colorectal neoplasia. We chose to examine this question by comparing two case groups relative to a control group. Such an analysis reveals both the case/case differences which we report in Table 3, as well as the relative risk estimates compared to a control group. The advantage of such analyses is that it allows you to examine case heterogeneity but also reveals the risk estimates. With case/case analyses only, it is entirely possible that the association between a risk factor and the tumor marker may be positively associated with disease in one group and negatively associated with disease in another group (e.g., the ORs straddle 1.0). The case/case difference will not reveal this which is an important distinction to make. This is why we used the control group.

5. The analysis of the data seems quite confusing. We agree with the reviewer in that the interesting comparisons to make are between the p53 negative cases and controls and p53 positive cases and p53 negative cases. This is exactly what we have estimated and reported. When using a polytomous model for 3 categories of outcomes, two comparisons (p53+ versus controls, and p53- versus controls) are direct outputs from the model. The third comparison (p53+ versus p53-) comes from subtracting the model parameters which we did with the corresponding confidence intervals. Therefore, it is not necessary to run separate models using p53 status as a reference category.

6. Why was an ordinal logistic regression model not used? This is an interesting question and the main reason we did not use an ordinal model is that even though the prevalence of p53 protein overexpression changed across the adenoma-carcinoma sequence the relationship between the risk factors and the different case groups did not. Interestingly, we also found the same risk factors associations that others have reported for invasive colorectal cancer. Thus, to improve statistical power, without affecting validity, we decided to
group the p53+ cases of adenomas, CIS, and IM together and group the p53-
cases of adenoma, CIS, and IM into a separate group.

I can be reached at the address below or by phone (212)-305-4915, fax (212) 305-
9413, or email (mt146@columbia.edu). Again, thank you for considering our paper
for publication in your journal.

Sincerely,

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