CORRESPONDENCE

DNA Methylation in Lung Cancer

TO THE EDITOR: Brock et al. (March 13 issue)[1] report that gene methylation, especially methylation of the *p16* and *CDH13* genes, was associated with early recurrence of stage I non–small-cell lung cancer (NSCLC). We previously evaluated the relationship between methylation of five genes and prognosis in 351 Japanese patients who underwent resection for NSCLC and found that only *p16* methylation was significantly related to a poor prognosis.[2] We analyzed our results again and found that methylation of both *p16* and *CDH13* was also associated with a poor prognosis in 199 patients with lung adenocarcinomas, even when these tumors were limited to stage I disease. We also reported that methylation of *p16* and *CDH13* was significantly less frequent in epidermal growth factor receptor (*EGFR*)-mutant tumors than in *EGFR* wild-type tumors, suggesting less involvement of methylation of these genes in *EGFR*-related tumorigenesis.[3] Our findings provide support for those of Brock et al. with regard to the prognostic significance of gene methylation in NSCLC.

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1. Brock MV, Hooker CM, Ota-Machida E, et al. DNA methylation markers and early recurrence in stage I lung cancer. N Engl J Med 2008;358:1118-28.
2. Toyooka S, Suzuki M, Maruyama R, et al. The relationship between aberrant methylation and survival in non-small-cell lung cancers. Br J Cancer 2004;91:771-4.
3. Toyooka S, Tokumo M, Shigematsu H, et al. Mutational and epigenetic evidence for independent pathways for lung adenocarcinomas arising in smokers and never smokers. Cancer Res 2006;66:1371-5.

TO THE EDITOR: An interesting finding by Brock and colleagues is that gene methylation in lymph nodes is associated with the recurrence of lung cancer; this implies that microscopically undetectable micrometastases can be detected by means of an assay for gene methylation. The validity of their multivariate analysis is in doubt, however, because of the inappropriate staging of lung cancer and the unusual differences in survival among patients with tumors of different T stages. Generally, the survival rates for patients with stage T1 lung cancer and those with stage T2 lung cancer differ significantly,[1] but the study by Brock et al. indicated that they were not significantly different. Perhaps incorrect staging or selection bias influenced their results.

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1. Rami-Porta R, Ball D, Crowley J, et al. The IASLC lung cancer staging project: proposals for the revision of the T descriptors in the forthcoming (seventh) edition of the TNM classification for lung cancer. J Thorac Oncol 2007;2:593-602.

THIS WEEK'S LETTERS

TO THE EDITOR: Brock et al. found that the methylation of a particular combination of genes had an astounding odds ratio of 25.25 for the recurrence of early-stage lung cancer. They examined the effect of each of these genes in three different types of tissues, performing at least 30 different statistical tests without a correction for multiple-hypothesis testing. A Bonferroni[1] correction applied to the data in Table 3 of the article indicates that only 2 of the 11 results originally shown to be significant remain significant.

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1. Manly KF, Nettletone D, Hwang JT. Genomics, prior probability, and statistical tests of multiple hypotheses. Genome Res 2004;14:997-1001.

TO THE EDITOR: Brock et al. suggest that their assay can identify micrometastases in lymph nodes and hence improve the staging of NSCLC. However, they report no data on the outcome when DNA methylation markers were detected in the primary tumor but not in the lymph nodes.

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THE AUTHORS REPLY: We acknowledge the confirmation by Toyooka et al. of our findings concerning methylation of *p16* and *CDH13* in lung adenocarcinomas in Japanese patients.[1] Park suggests that we may have inappropriately staged the disease in our patients, since the survival rates among patients with T1 lesions and patients with T2 lesions were not significantly different. In our study, the adjusted hazard ratio for the risk of recurrence of a stage T2 lung cancer tumor, as compared with the risk of recurrence of a stage T1 cancer, was 1.71 (95% confidence interval, 0.86 to 3.41; P=0.13), which is similar to that reported in recent studies.[2,3]

Riaz suggests that our results require correction for multiple-hypothesis testing. However, the validity of our findings would be diminished if gene methylation were inconsistent in different tissues in their anatomical context. Our findings were consistent in tumors and lymph nodes. Moreover, corrections for multiple testing were not required, since we chose genes on the basis of hypotheses generated from earlier studies that indicated the importance of methylation of these genes in lung cancer. We did not embark on an investigation with the use of a large set of potentially error-ridden possibilities (i.e., genes) based primarily on P values. However, we presented the estimated odds ratios and confidence intervals for all seven genes as the main currency of our inferences. In response to Tezcan, the methylation of the genes we studied does have biologic consequences, and the reversal of methylation may open new possibilities for treatment.

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1. Toyooka S, Tokumo M, Shigematsu H, et al. Mutational and epigenetic evidence for independent pathways for lung adenocarcinomas arising in smokers and never smokers. Cancer Res 2006;66:1371-5.
2. Goya T, Asamura H, Yoshimura H, et al. Prognosis of 6644 resected non-small cell lung cancers in Japan: a Japanese Lung Cancer Registry study. Lung Cancer 2005;50:227-34.
3. Pfannschmidt J, Muley T, Bülzebruck H, Hoffmann H, Dienemann H. Prognostic assessment after surgical resection for non-small cell lung cancer: experiences in 2083 patients. Lung Cancer 2007;55:371-7.

Abacavir Hypersensitivity

TO THE EDITOR: The Prospective Randomized Evaluation of DNA Screening in a Clinical Trial (PREDICT-1) by Mallal et al. (Feb. 7 issue)[1] showed that screening for HLA-B*5701 resulted in a significant reduction in the rate of hypersensitivity reactions (from 7.8% to 3.4%) among patients who were positive for the human immunodeficiency virus (HIV) and were receiving abacavir. However, because of methodologic limitations, this study will only modestly alter clinical practice. In the