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“Genome-Wide Hypomethylation and Cancer Risk” --- Letter

To the Editor

Brennan and Flanagan’s recent publication in *Cancer Prevention Research* describing a meta-analysis of blood methylation and cancer risk is an outstanding contribution to this burgeoning field (1). The authors discuss a wide-ranging set of important issues and make numerous salient points regarding the development of methylation-based blood biomarkers for cancer risk management. In particular, their finding that global 5-methylcytosine (5meC) based studies have shown a significant cancer risk association, while studies analyzing individual repetitive elements (RE) (including *LINE-1*) have not, is notable. In this context, I would like to clarify two important issues raised in the review. The first regards the method and input DNA requirement needed for measurement of 5meC, and the second involves the association between 5meC and RE methylation in blood and in tumor tissues. In the first case, the authors state that, in our study of blood methylation and breast cancer risk (2), we used HPLC to measure 5meC, and that this assay requires large amounts of input DNA, making it unsuitable for population-based studies. In fact, our assay used HPLC coupled with mass spectrometry (i.e. LC-MS), greatly increasing assay sensitivity, and specifically measuring 5-methyl-2 -deoxycytidine (5mdC) (2,3). We validated the LC-MS assay down to low ng amounts of input DNA, although in practice we typically utilize 800 ng gDNA (3). Even this higher amount is on the order of, and often less than, the amount of gDNA used for sodium bisulfite-based DNA methylation analyses (including RE pyrosequencing). Additionally, an improved LC-MS assay that is more precise, reduces gDNA input, and significantly reduces assay cost, has been reported (4). Thus, LC-MS analysis of 5mdC remains a viable approach for population-based methylation studies. A second issue raised in the review pertains to the perceived absence of published data correlating 5mdC with *LINE-1* methylation. In this context, the authors correctly cite our study, wherein we failed to observe a correlation using blood DNA (2). However, in contrast to blood, we observed significant correlation between 5mdC and *LINE-1* methylation in tumor tissues (5). 5mdC also correlated with methylation of the *Alu* RE but not with the *Satellite-* RE in our tumor study (5). Thus, 5mdC does in fact correlate with methylation of specific REs in tumor tissues, but this should be experimentally validated in the system under study. Nevertheless, I agree with the authors’ well-reasoned point that RE methylation may be a poor surrogate for 5mdC in normal blood.

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