Letter to the Editor

Cancer Prevention Research

Genome-Wide Hypomethylation and Cancer Risk—Letter

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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 LINE1) 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 repetitive element 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 high-performance liquid chromatography (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., liquid chromatography/mass spectrometry; LC-MS), greatly increasing assay sensitivity, and specifically measuring 5-methyl-2′-deoxycytidine (5mdC; ref. 2, 3). We validated the LC-MS assay down to low nanogram amounts of input DNA, although in practice we typically use 500 ng to 1.0 μg 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 repetitive elements pyrosequencing). In addition, 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 LINE1 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 LINE1 methylation in tumor tissues (5). 5mdC also correlated with methylation of the *Alu* repetitive elements but not with the *Satellite-α* repetitive elements in our tumor study (5). Thus, 5mdC does in fact correlate with methylation of specific repetitive elements in tumor tissues, but this should be experimentally validated in the system under study. Nevertheless, I agree with the authors' well-reasoned point that repetitive elements methylation may be a poor surrogate for 5mdC in normal blood.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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