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Cancer Prevention Research

Letter to the Editor

Genome-Wide Hypomethylation and Cancer Risk—Response

Kevin Brennan and James M. Flanagan

We thank Dr. Karpf for his interest in our recent review in *Cancer Prevention Research* describing epigenetic epidemiology and the search for biomarkers of cancer risk using genome-wide methylation detectable in blood DNA (1). We recently reported a candidate methylation marker for breast cancer risk within the ATM gene using 3 prospective cohorts (2). Within this study, we found no evidence for an association between breast cancer risk and LINE1 methylation in blood DNA. In our review, we outlined several important limitations in many of the studies published to date including the low interindividual variability of some assays and the use of retrospectively collected blood samples in which the presence of cancer is likely to confound any risk analysis. Regarding the DNA requirements for high-performance liquid chromatography (or liquid chromatography/mass spectrometry; LC-MS) based methods, Dr. Karpf states that they routinely use 500 ng to 1 μg, but have used 1 μg (3), while others use up to 3 μg (4). While, in theory, lower amounts can be used, in practice these larger amounts are typically used. In our experience, large prospective cohort studies that we have collaborated with are rightly unwilling to provide more than 250 ng, or 500 ng at most, of genomic DNA from their precious resources. We routinely add 250 to 500 ng DNA to the sodium bisulphite conversion reaction, sufficient for 30 different methylation loci, requiring 8 to 16 ng DNA for each pyrosequencing assay. Alternatively, 500 ng DNA can be used to measure DNA methylation using genome-wide arrays. The correlation between individual repetitive elements and total methylcytosine (5meC) in tumor DNA is not in question, particularly when the range of biologic variation (range 25%–75%; ref. 3) greatly exceeds the technical variation of the pyrosequencing assay (2%–3%; ref. 2). We have found that the biologic variation between individuals in LINE1 methylation in blood (IQR of 1.8%–3.7%) using prospectively collected cohorts did not significantly exceed the technical variation in duplicate samples from the cohorts (1.8%), presented as an intraclass correlation coefficient between duplicate samples; ICC = 0 (95% confidence interval, 0–0.61; ref. 2). In support of this, a recent study showed no evidence of an association with cancer risk and 5meC as measured by LC-MS, and no correlation between LINE1 and 5meC in blood DNA or in normal colonic mucosal DNA ($n = 226$ subjects; ref. 5). While our meta-analysis of the numerous studies of repetitive element methylation suggests that there is no true association with cancer risk, the association between 5meC and cancer risk remains to be replicated in an appropriately sized prospective cohort study with incident cancer cases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Authors' Affiliation: Epigenetics Unit, Department of Surgery and Cancer, Imperial College London, United Kingdom

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