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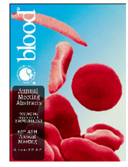

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

803.EMERGING TOOLS, TECHNIQUES AND ARTIFICIAL INTELLIGENCE IN HEMATOLOGY

Genome-Wide Methylation Sensitive Elements Demarcate Targetable Regulatory Elements for Controllable Gene Activation

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There are multiple published studies correlating DNA methylation with gene expression, but to date, most are associative in nature without precise localization of causal elements. Multiple CpG destinations have been described with inconsistent evidence supporting their causal association with transcription. This has hampered adoption and use of methylation signatures as biomarkers or for targeted therapies. This has had consequent effects in hindering mechanistic understanding in how global demethylation agents lead to responses in some patients, but not others. With this state of the literature in mind, we set out to better understand and locate causal methylation regulatory loci genome-wide.
We hypothesized that causal methylation regulatory elements possess heightened sensitivity to regulatory signals such as "demethylate"; signals that can be introduced using hypo-methylating agents (HMAs) typically used in treatment of Myelodysplastic syndromes and Acute Myeloid Leukemia. We thus analyzed 19 whole-genomic bisulfite sequenced sample pairs (pre/post-demethylation in patients, cell lines, and in murine in vivo samples) and identified previously undescribed DNA methylation sensitive elements throughout the genome, which we call Methylation Mesas (MMs) based on the profile shape observed in naïve and post-HMA treated sample. These narrow-width MMs range typically between 45-300bp wide and are present throughout the genome with greater abundance in noncoding and intragenic regions than in promoter-CpG islands. We also demonstrate that MM show an up to 90% concordant overlap with primed and active histone marks by means of a compiled histone mark footprint map using 60 human and 59 murine ENCODE datasets for each respective species, suggesting a functional role for MMs as regulatory elements with transcriptional potential.
While HMA's enable identification of MM's genome-wide, their mode of action renders them unsuitable for ascertaining the direct transcriptional potential of any singular locus. Therefore, in order to ascertain the causal role of MM for transcriptional regulation, we developed a fine-resolution targeted demethylation technology(CRISPR-DiR), leveraging our previously published findings that DNA methyltransferase I (DNMT1) can be blocked by DNMT1-interacting RNAs (DiR). Compared with other targeted demethylation technologies with wider effect windows (eg. CRISPR-TET1), CRISPR-DiR shows superior locus specificity thus serving as an ideal tool to identify causal regulatory elements. Using tumor suppressor p16 as an example, we

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elucidated that demethylation of a single MM in the first exon is sufficient to trigger locus and distal chromatin rewiring events that natively initiate expression to a significantly greater extent than promoter CpG island targeting alone. Similar CRISPR-DiR activation is also observed in four additional, natively silenced tumor suppressor genes, one of which has no annotated CpG island.

In summary, we have identified a previously undescribed narrow-width DNA methylation sensitive regulatory element, consistent across all epigenomes investigated, located largely independent of the promoter CpG island dogma, yet initiating gene transcription more potently. Identification of said elements paves the way for future studies to better understand the mechanisms of how mutations in DNA methyltransferases lead to disease, how global hypomethylating agents can lead to a response in diseases such as MDS and AML but can also activate oncogenes, and finally development of targeted therapies.

Disclosures No relevant conflicts of interest to declare.

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