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POSTER ABSTRACTS

503.CLONAL HEMATOPOIESIS, AGING AND INFLAMMATION

DNA Methylation Instability: A Novel Biomarker for Aging, Clonality, and Cancer

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Background:
Clonal hematopoiesis (CH) is an age-related condition defined by the over-representation of blood cells derived from a single clone. CH has been associated with an increased risk of leukemia development, adverse cardiovascular events, and all-cause mortality. There has been great emphasis on investigating CH at the genomic level, yet the epigenetic factors that regulate the development and progression of pre-malignant clones haven't been extensively studied.
Methods:
Here, we develop a novel framework that employs DNA methylation (DNA-M) to detect abnormal clonal expansion of hematopoietic cells. DNA-M profiles of >1500 blood samples from healthy, young persons (age = 18) were interrogated to identify CpG sites with highly consistent methylation levels across individuals. DNA methylation instability (DMI), which we define as deviation in methylation levels at the identified stable methylation sites (SMSs), was evaluated in diverse healthy and cancer cohorts to investigate how epigenetic mechanisms may affect cellular functions resulting in clonality.
Results:
Characterization of SMSs.
SMSs were found to be predominantly unmethylated and significantly enriched within CpG islands (OR: 13.8, $p < 2 \times 10^{-16}$) and gene promoter regions (OR: 4.8, $p < 2 \times 10^{-16}$). A gene ontology enrichment analysis implicated the corresponding genes in vital cellular processes such as DNA repair, damage response, and cell cycle checkpoints ($p < 0.002$). Consistently low methylation levels at SMSs were observed across major blood cell types. Moreover, analysis of a methylome cell atlas derived from whole genome bisulphite sequencing (WGBS) of 40 healthy human tissues revealed that SMSs remain stably unmethylated in the vast majority of tissues, suggesting broad and strict regulation at those sites.
DMI in overt blood cancers.
Interrogation of SMS methylation levels across diverse control and cancer datasets revealed significantly higher destabilization in cancer samples (Fig. 1, $p < 2 \times 10^{-16}$). Moreover, longitudinal profiling of bone marrow samples from AML patients (n = 4, four time points each) indicated that DMI levels follow the clonal burden patterns expected during treatment. Analysis of serial dilutions of AML cell lines in cord blood further corroborated the link between DMI levels and malignant cell fractions ($p < 2 \times 10^{-16}$).
DMI in non-cancer cohorts.
Given the link between DMI and clonality, we measured DMI in blood samples of three independent aging cohorts of individuals without a malignancy (n = 1751, age 14-101) and observed a positive correlation with age (r = 0.25, 0.38, 0.4; $p < 7 \times 10^{-11}$). These results are in accordance with the increasing incidence of CH with age and suggest that DMI, much like genomic mutations, may causally precede the development and progression of various age-related diseases. CH has previously been associated with adverse cardiovascular outcomes. In a competing risk analysis of 64 cardiogenic shock patients (CH+ = 31, CH- = 33), DMI was better able to stratify patients into risk groups than genomics-based CH detection (Kaplan-Meier analysis, CH: p = 0.46, DMI: p = 0.01). These results indicate that DMI is significantly associated with mortality in high-risk patients.
DMI and gene regulation.
WGBS of AML and healthy control samples revealed that significantly destabilized SMSs in AML ($p < 7 \times 10^{-6}$) near transcriptional start sites reflect broader hypermethylation of promoter regions. Transcriptome profiling of age-stratified controls (n = 755) and AML samples (n = 151) confirmed a gradual decrease in expression for many of the implicated genes. Notably, genes known to suppress cell proliferation and promote apoptosis such as BASP1 were implicated (Fig. 2, r = -0.20, $p < 2 \times 10^{-16}$),

as well as novel genes that have not previously been associated with age-related gene silencing and leukemia development. These findings further emphasize that DMI reflects a gradual process that precedes disease.

Conclusion:

Our results suggest that the development of clonality is not merely a genomic phenomenon and that the gradual silencing of specific genes via DMI in their promoter regions may result in positive selection and clonal dominance. This work has significant implications for elucidating the molecular mechanisms underlying age-related diseases, early detection of cancer, and the development of novel biomarkers and therapeutic strategies against hematologic malignancies.

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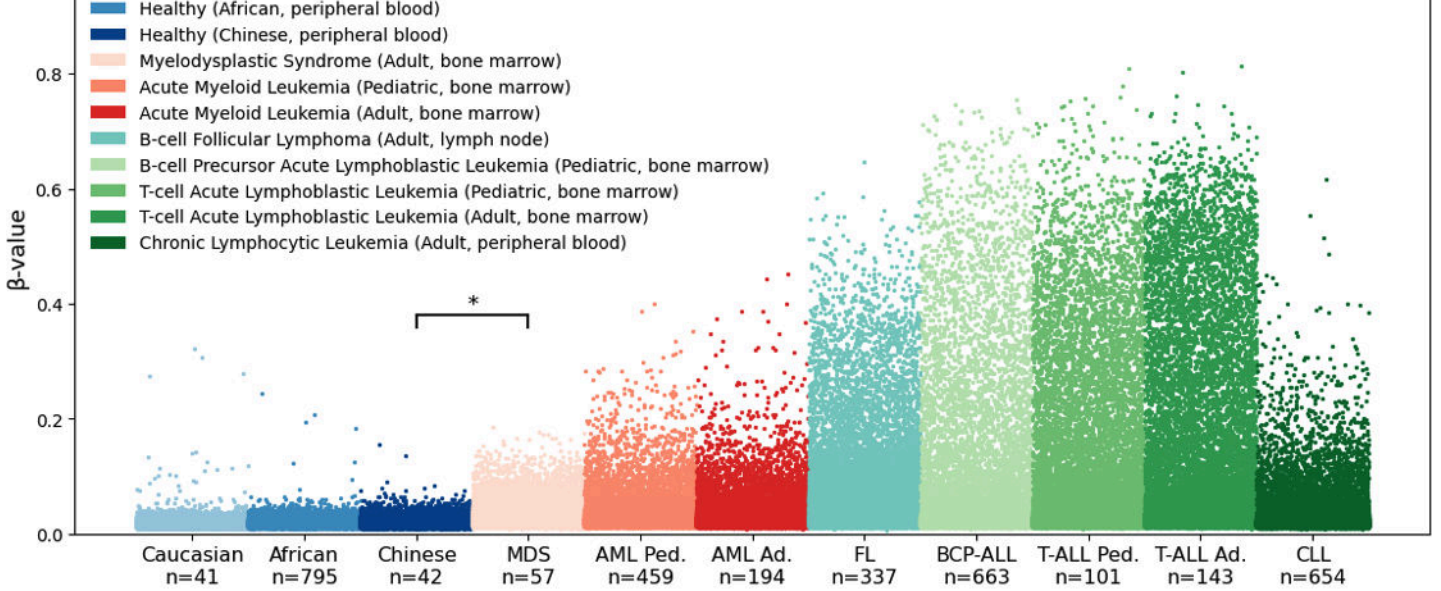

Figure 1: Methylation levels at stable methylation sites in healthy and cancer cohorts. Mean β-values of SMSs across individuals. Each point represents a single SMS (n=27,850). * Wilcoxon test, $p < 2.2 \times 10^{-16}$

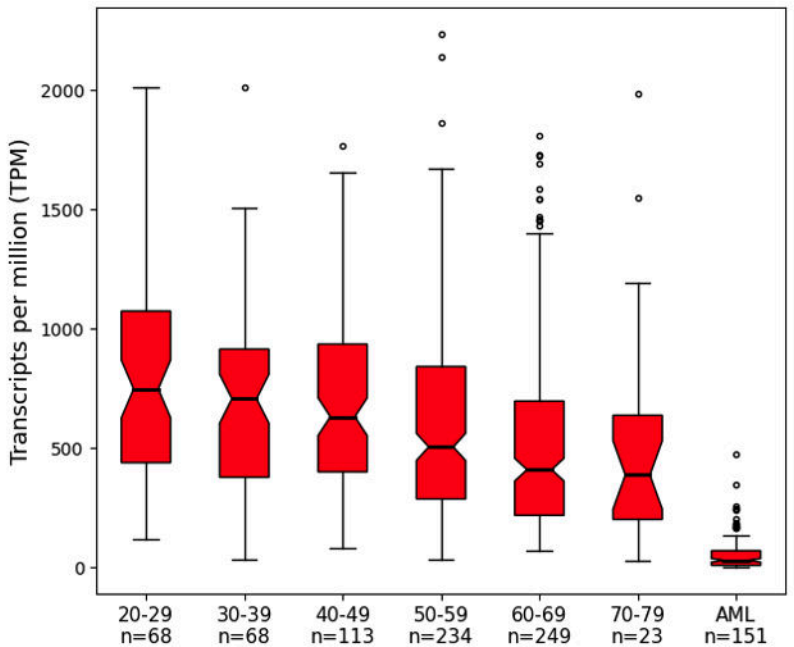

Figure 2: Expression of BASP1 in age-stratified healthy individuals and AML patients. Transcripts per million (TPM) for BASP1.