Long-read sequencing identifies and refines genome-wide methylation signals in multiple myelomas

Poster # **8157W**

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Background

- **Multiple myeloma** is a blood cancer that originates in plasma cells. Multiple myelomas are very heterogeneous, heavily influenced by the exact somatic, copy-number, and epigenetic factors that are part of disease progression.[1]

- **Somatic epigenetic defects** have been observed in multiple myelomas with two primary epigenetic changes[2]:
  - **Global hypo-methylation** is a genome-wide reduction in 5mC methylation. This can lead to genomic instability, potentially causing the expression of genes that are not normally expressed in healthy cells.
  - **Local hyper-methylation** is a localized increase in 5mC methylation, which tends to target promoter regions (CpG islands) upstream of gene bodies. This can lead to gene silencing of tumor suppressor and other genes that are normally expressed in healthy cells.

- **Long-read sequencing (PacBio HiFi)** simultaneously detects genomic sequence (ACGT) and epigenetic modifications (5mC, 5hmC, and 6mA), making it a useful assay for detecting genetic and epigenetic variation in tumor samples.

Methods

1. Sequence tumor samples using long-read sequencing
   - 10 tumor samples – multiple myelomas cell lines
   - 45 normal samples – blood-derived cell lines (e.g., HG002) used as a control population for measuring statistical differences in methylation state
   - Genomic sequences with methylation readouts for 5mC, 5hmC, and 6mA

2. Process all datasets using standard workflows for alignment, variant calling, and epigenetic pileup
   - Alignment – pbmm2 for alignment to GRCh38 reference genome
   - Variant calling and phasing – small (DeepVariant), structural (sawfish), and somatic (DeepSomatic) variants jointly phased together (HiPhase)
   - 5mC methylation pileup – generated using MethBat workflow

3. Analyze 5mC methylation status using the MethBat toolkit
   - "Signature" mode – *De novo* identify regions with differential methylation patterns between two groups of samples (e.g., case/control).
   - "Compare" mode – Quantify the differential methylation patterns in pre-defined regions (e.g., CpG islands) between two groups of samples.

Global hypomethylation in multiple myelomas

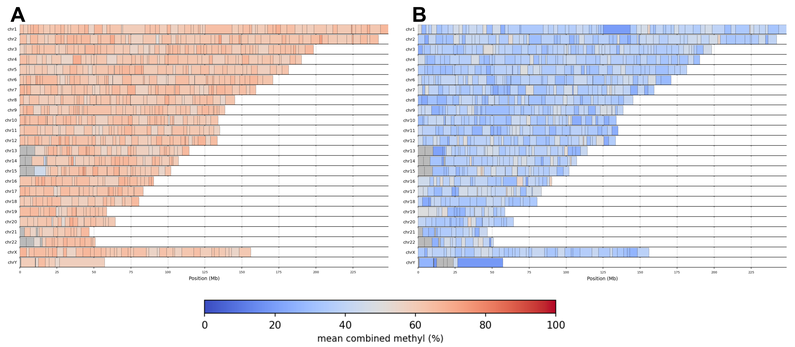


Figure 1. **Karyograms of methylation**. These karyograms show the average methylation of control sample HG002 (**A**) and a multiple myeloma cell line, DP-6 (**B**), across the genomic bands. Global hypomethylation (**blue**) is visually apparent in DP-6 compared to the control sample which is generally methylated (**red**).

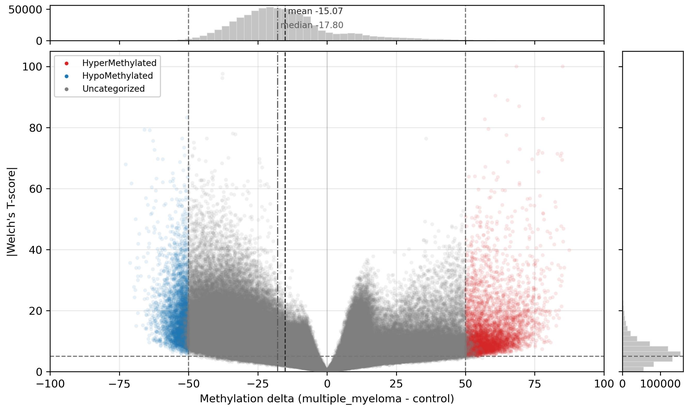


Figure 2. ***De novo*** **segmentation of multiple myeloma cell lines v. controls**. Each of the above points is a region created by segmenting the genome based on the difference between the multiple myeloma and the control methylation states. Regions with >50% methylation delta and with statistically significant differences (Welch's T-score) are labeled as hyper- or hypo-methylated. The methylation delta median is -17.80, indicating that the multiple myeloma datasets have a lower methylation score on average genome-wide, reflecting the known global hypomethylation pattern of this data type.

Local hypermethylation of CpG islands

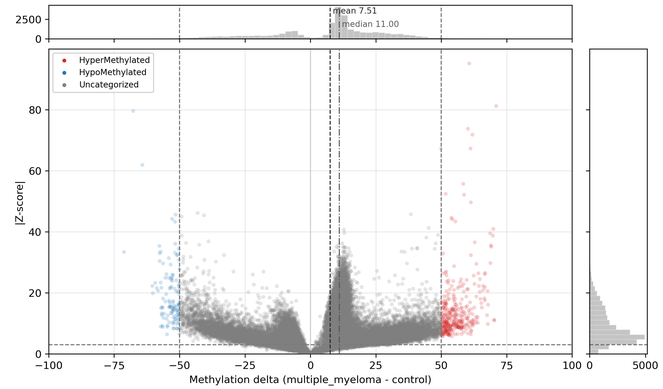


Figure 3. **Multiple myeloma cell lines v. controls in established CpG island regions**. Each of the above points is a single CpG island region showing the difference in mean methylation between the multiple myeloma and control datasets. Regions with >50% methylation delta and with statistically significant differences (Z-score) are labeled as hyper- or hypo-methylated. In contrast to the genome-wide signal, the median methylation delta across all CpG islands is +11.00, indicating that the multiple myeloma datasets have local hypermethylation of the CpG island regions.

Conclusions

Long-read sequencing detected both known epigenetic signals in multiple myelomas:

- Genome-wide hypo-methylation causing genomic instability and potentially over-expression of genes not normally expressed in blood cells
- Local hyper-methylation of promoter regions, potentially silencing repair mechanisms in tumor suppressor genes

The MethBat toolkit is useful for epigenetic analysis of tumor samples:

- *De novo* segmentation of the genome into regions of interest
- Comparative analyses of pre-defined genomic regions like CpG islands

Find MethBat on GitHub!

References

1. Schavgoulidze, Anaïs, et al. "Multiple myeloma: heterogeneous in every way." *Cancers* 13.6 (2021): 1285.
2. Muylaert, Catharina, et al. "Aberrant DNA methylation in multiple myeloma: A major obstacle or an opportunity?." *Frontiers in oncology* 12 (2022): 979569.

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