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Investigating Hypermutation in the Inactive X-Chromosome [2425MED1006]

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INVESTIGATING HYPERMUTATION IN THE INACTIVE X-CHROMOSOME: A CANCER DETECTION KEY

INTRODUCTION

In female mammals, X-chromosome inactivation (XCI) silences one X chromosome via DNA methylation, resulting in **inactive ChrX (X_i) and active ChrX (X_a)**. In cancer, X_i accumulates more somatic mutations (up to 4 times) than autosomes (**X_i hypermutation**) due to its late replication and heterochromatic state (Jäger et al., 2013).

This unique mutational profile positions X_i hypermutation as a promising **cancer biomarker**, though distinguishing X_i from the X_a remains a key challenge for clinical translation.

PURPOSE

- **Distinguish** the inactive X-chromosome, X_i from X_a by analyzing haplotype-specific patterns (HP=1 vs. HP=2)
- **Quantify** methylation differences between X_i and X_a to test for Xi-specific hypermutation

Image2: X-Chromsome

METHODOLOGY

1. Variant Calling and Methylation Detection

Germline small variants were identified using Clair3 on ONT (Oxford Nanopore Technologies) sequencing data to detect heterozygous SNPs for haplotype phasing. Methylation was then detected by passing DNA through a nanopore and measuring ionic current changes to identify modified bases (e.g., 5-methylcytosine) via distinct current signatures.

2. Haplotype Classification and BAM File Processing

Methylation calls from BAM files were processed with ClairS-TO to call somatic variants and separate them into haplotypes (HP1 and HP2) in Acute Lymphoblastic Leukemia (ALL) samples SYQ, XL, and LJM, leveraging its phasing capabilities. Modkit was then used to extract and assign methylation calls to these haplotypes for allele-specific analysis. The separated haplotypes were visualized in IGV to confirm methylation differences.

3. Methylation Rate Calculation and Analysis

Methylation rates per haplotype were calculated as the ratio of methylated to total calls using a Python pipeline. Rates were aggregated across promoter-associated CpG islands, analyzed to identify the inactive X chromosome, and visualized with histograms and KDE plots.

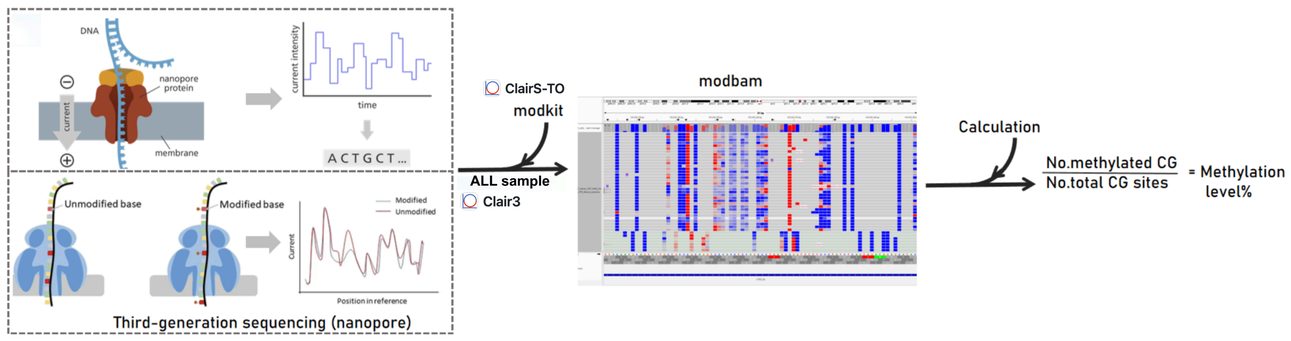

Image1: Workflow for X chromosome methylation analysis

RESULTS

BAM File Processing

In the G6PD promoter region, IGV for SYQ shows track 1 (HP1) mostly red (unmethylated) and track 2 (HP2) mostly blue (methylated), indicating HP2 as the inactive X chromosome (Xi) in SYQ, consistent with Xi hypermethylation in XCI. LJM shows a similar pattern with significant red in HP1 and blue in HP2, also indicating HP2 as the Xi. In contrast, XL follows a similar trend but has some blue in HP1, suggesting less distinct methylation differences between haplotypes.

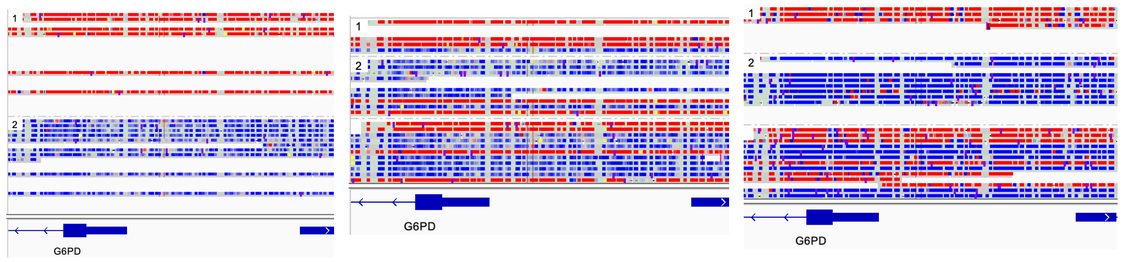

Image3: IGV visualization of methylation in the G6PD promoter region in SYQ, XL, LJM)

Methylation Rate Distribution

Methylation rate distributions for ChrX promoter CGIs (TSS and H3K4me3) in SYQ, XL, and LJM were plotted (Image 4). All distributions are bimodal, with peaks near 0 and 1, reflecting XCI in promoter CGIs where the X_i is hypermethylated and the X_a is hypomethylated. In the plots, HP1 is blue and HP2 is yellow.

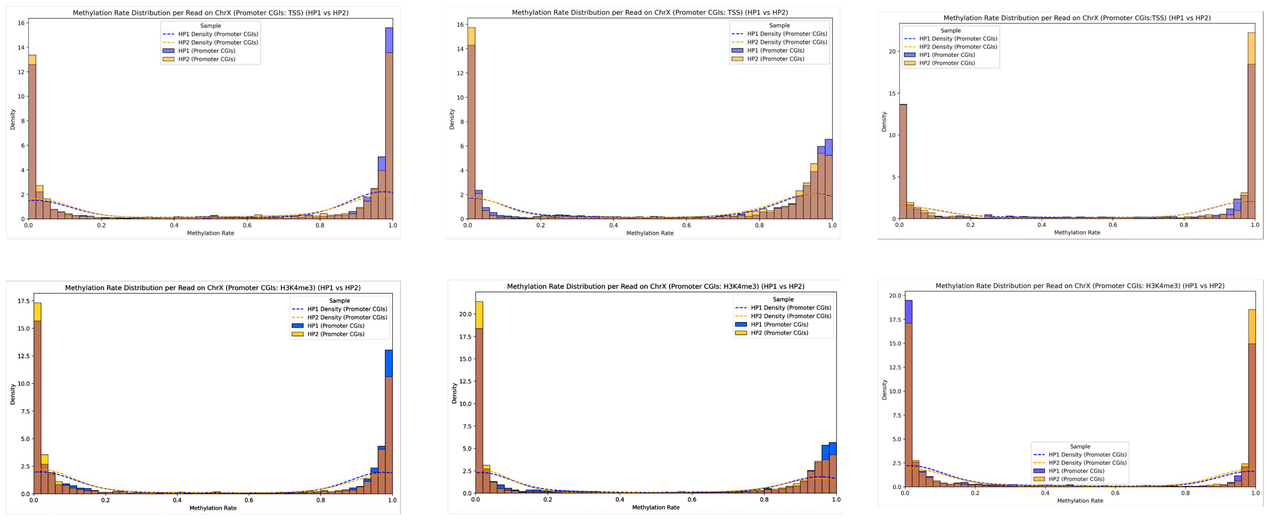

Image4: Methylation rate distribution plots for ChrX promoter CGIs (TSS and H3K4me3) in SYQ, XL, and LJM

For SYQ, blue (HP1) peaks at 1 (right) and yellow (HP2) at 0 (left), confirming HP1 as the X_i. LJM shows a similar trend, but blue (HP1) is less pronounced at 1, indicating less significant hypermethylation.
But for XL, the pattern reverses: yellow (HP2) peaks at 1 and blue (HP1) at 0, suggesting HP2 as the X_i.

CONCLUSION

Our study identifies the inactive X chromosome (X_i) in SYQ, LJM, and XL via methylation patterns in promoter CGIs, with HP2 as the Xi in SYQ and LJM, though XL shows less distinct differences and patterns can reverse, indicating an unverified effect. This may be due to (1) poor haplotype classification from ClairS-TO's complex workflow and (2) insignificant methylation in some promoters. Future work could enhance mutation calling, analyze HP1/HP2 at more loci, or use histone profiling instead of methylation rates to better distinguish X_i and X_a.

REFERENCES

Jäger, N., Schlesner, M., Jones, D. T., Raffel, S., Mallm, J. P., Junge, K. M., Weichenhan, D., Bauer, T., Ishaque, N., Kool, M., Northcott, P. A., Korshunov, A., Drews, R. M., Koster, J., Versteeg, R., Richter, J., Hummel, M., Mack, S. C., Taylor, M. D., Witt, H., ... Eils, R. (2013). Hypermutation of the inactive X chromosome is a frequent event in cancer. Cell, 155(3), 567–581. https://doi.org/10.1016/j.cell.2013.09.042