Integrated epigenomic insights into breast cancer phenotypes and identification of early drivers

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Background

Breast cancer is a leading cause of death and is the most frequently diagnosed cancer worldwide[1]. It is highly heterogeneous, divided into subtypes that associate with different prognoses and treatments. Triple-negative breast cancer (TNBC) is highly aggressive, has the worst outlook, and is more frequent in younger women with limited treatment options[2].

Earlier stratification of patients can lead to earlier treatment decisions and better outcomes. The epigenome is a potent source of early, detectable biomarkers[3] that may inform on TNBC development and disease trajectory. Integration of public epigenomic and transcriptomic can identify novel biomarkers that may inform on subtyping and treatment sensitivity.

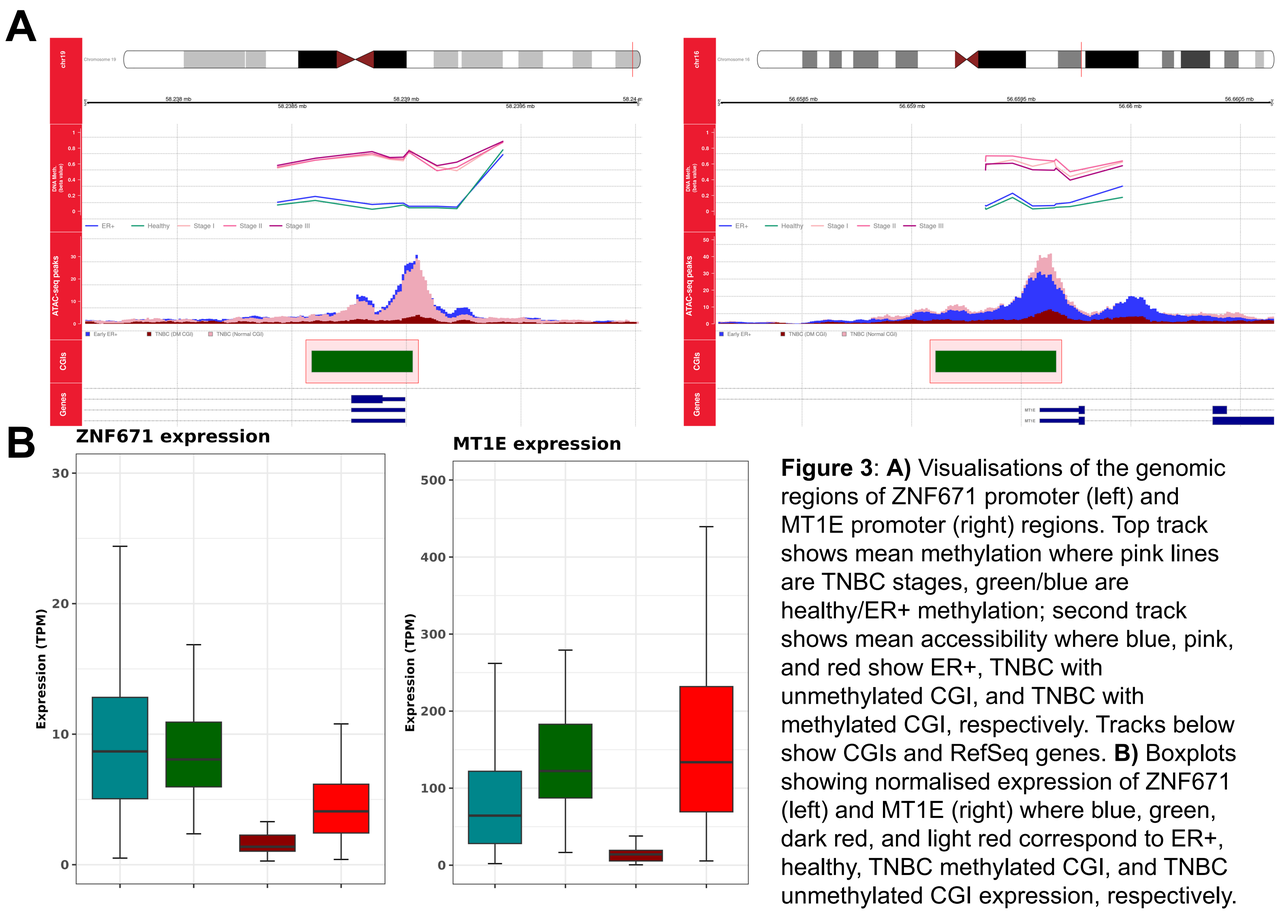

Figure 3: **A)** Visualisations of the genomic regions of ZNF671 promoter (left) and MT1E promoter (right) regions. Top track shows mean methylation where pink lines are TNBC stages, green/blue are healthy/ER+ methylation; second track shows mean accessibility where blue, pink, and red show ER+, TNBC with unmethylated CGI, and TNBC with methylated CGI, respectively. Tracks below show CGIs and RefSeq genes. **B)** Boxplots showing normalised expression of ZNF671 (left) and MT1E (right) where blue, green, dark red, and light red correspond to ER+, healthy, TNBC methylated CGI, and TNBC unmethylated CGI expression, respectively.

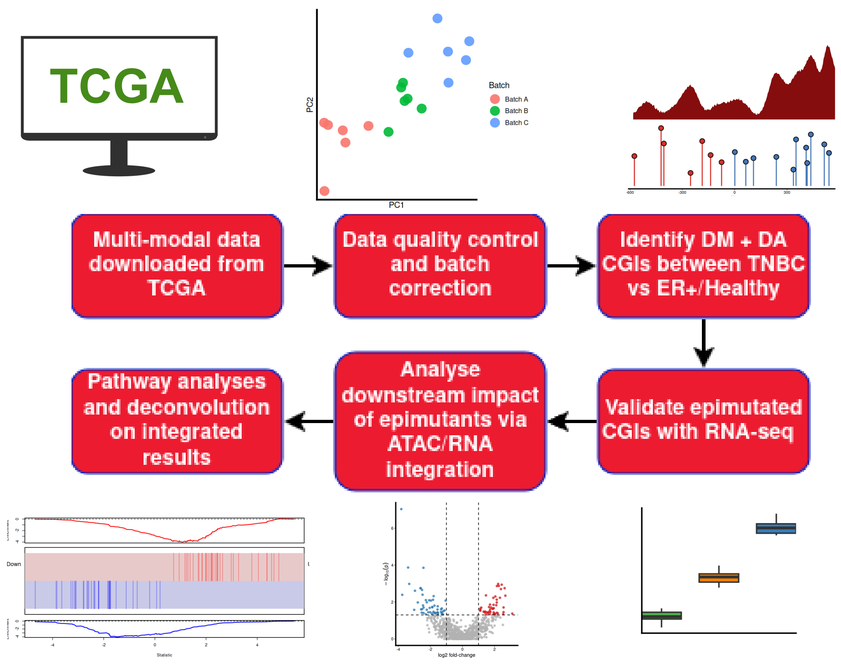

Figure 1: Brief epigenomic analysis workflow overview

Results

Results demonstrated a distinct ATAC-seq signal between TNBC and estrogen receptor (ER+) expressing samples (figure 2), moreso than DNA methylation. Integration of DNA methylation and ATAC-seq data revealed several novel potential drivers frequently present in TNBC samples, including the transcription factor ZNF671 and the metallothionein MT1E (figure 3).

Downstream differential analysis between ZNF671 methylated/unmethylated samples shows increased expression and CGI-associated promoter accessibility of PARP11, cytokines, and immune signalling pathways in ZNF671-unmethylated samples (figure 4A-B) with deconvolution of bulk RNA-seq samples suggests increased immune cell composition (figure 4C).

Data & Methods

Using patient matched DNA methylation (450K arrays; n=896), chromatin accessibility[4] (ATAC-seq; n=74), and expression data (RNA-seq; n=1,086) from The Cancer Genome Atlas (TCGA)[5] we performed CpG island (CGI) focused analyses to identify regions with increased accessibility and decreased methylation (or vice versa) and validated functional effects on associated genes using RNA-seq data (figure 1).

Downstream effects of specific epimutations were further characterised by integration ATAC-seq and RNA-seq data in samples where both modalities were available, performing differential analyses and enrichment of gene sets (figure 4).

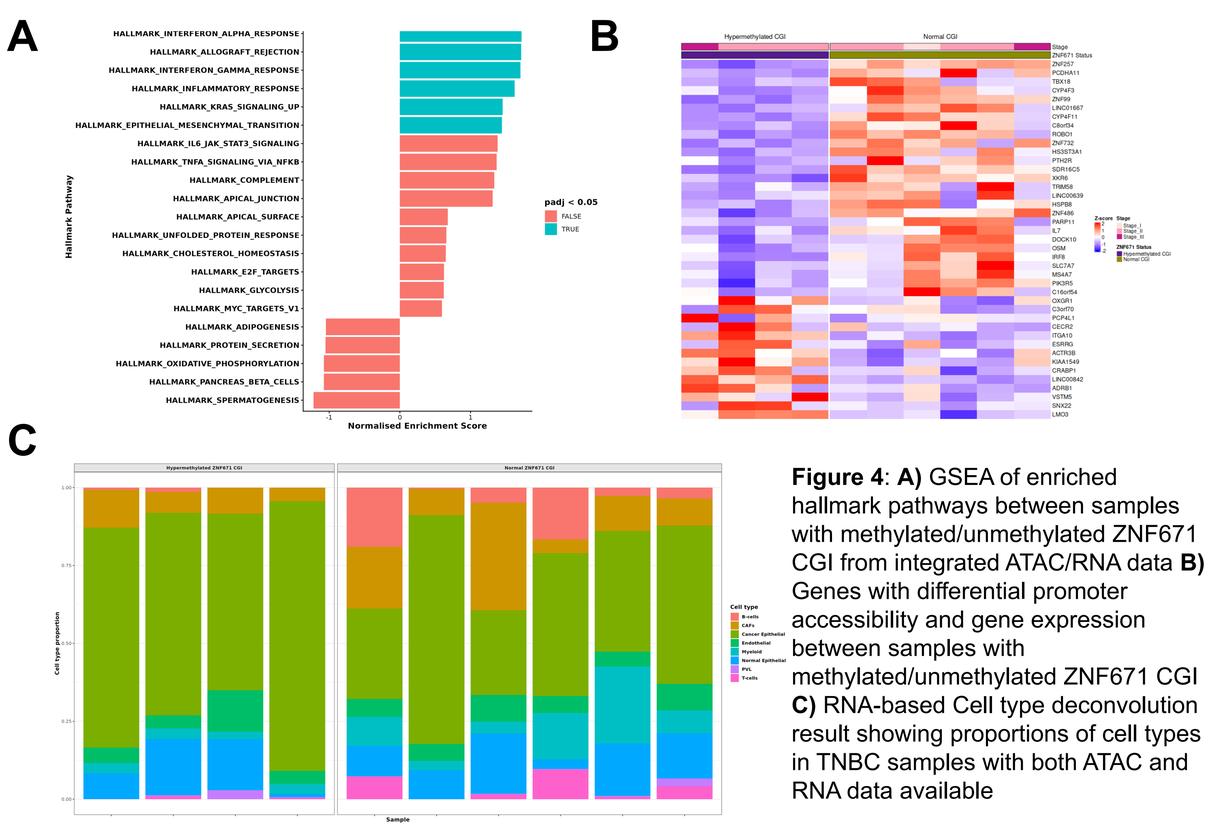

Figure 4: **A)** GSEA of enriched hallmark pathways between samples with methylated/unmethylated ZNF671 CGI from integrated ATAC/RNA data **B)** Genes with differential promoter accessibility and gene expression between samples with methylated/unmethylated ZNF671 CGI **C)** RNA-based Cell type deconvolution result showing proportions of cell types in TNBC samples with both ATAC and RNA data available

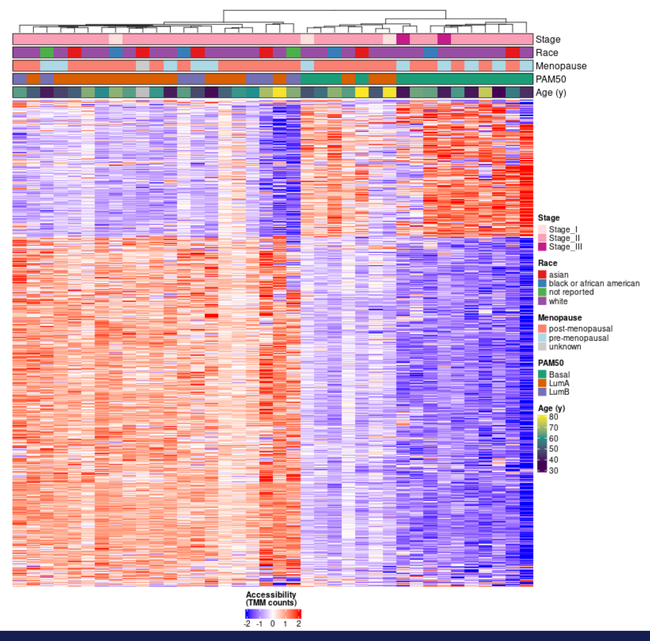

Figure 2: Heatmap of top 5,000 CGIs with most variable accessibility across all samples. Blue indicates a decrease in accessibility; red indicates a increase in accessibility

Discussion

ZNF671 is a promising target for further research and validation: it may inform on potential insensitivity to immunotherapies and PARP inhibitors. ZNF671, MT1E, and other identified epigenomic biomarkers may have prognostic use in earlier stratification of breast cancer patients. Epigenomic analysis is a promising field for future biomarker discovery in cancer.

References

1. Bray, F. et al. (2024) 'Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries', CA: A Cancer Journal for Clinicians, 74(3), pp. 229–263. Available at: https://doi.org/10.3322/caac.21834 2. Kim, C. et al. (2018) 'Chemoresistance evolution in triple-negative breast cancer delineated by single-cell sequencing', Cell, 173(4), pp. 879–893.e13. Available at: https://doi.org/10.1016/j.cell.2018.03.041 3. Vietri, M.,Teresa et al. (2021) 'DNA methylation and breast cancer: A way forward (review)', Int J Oncol; International Journal of Oncology, 59(5), pp. 98. Available at: https://doi.org/10.3892/ijo.2021.5278 4. Corces, C. et al. (2018) 'The chromatin accessibility landscape of primary human cancers', Science, 362(6413), pp. eaav1898. Available at: https://doi.org/10.1126/science.aav1898 5. Koboldt, D.C. et al. (2012) 'Comprehensive molecular portraits of human breast tumours', Nature, 490(7418), pp. 61–70. Available at: https://doi.org/10.1038/nature11412