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Exploring the Role of Epigenetic Modifications in Regulating Gene Expression During Development and Disease Progression: A Case Study of Malaria in Female Adult Wistar Rats

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Abstract

EPIGENETIC modifications play crucial roles in regulating gene expression during both normal development and pathological conditions. This study investigates the impact of malaria infection on epigenetic mechanisms in female adult Wistar rats, focusing on DNA methylation, histone modifications, and chromatin remodeling patterns. Through comprehensive analysis of epigenetic landscapes before and after *Plasmodium* infection, we demonstrate significant alterations in host gene expression profiles that contribute to disease pathogenesis and immune responses. Our findings reveal that malaria infection induces widespread changes in the host epigenome, affecting genes involved in immune function, metabolism, and cellular stress responses. This research contributes to understanding how infectious diseases manipulate host epigenetic machinery and provides insights for potential therapeutic interventions.

Keywords: Epigenetics, malaria, gene expression, DNA methylation, histone modifications, Wistar rats

Introduction

EPIGENETIC modifications represent a fundamental layer of gene regulation that operates without altering the underlying DNA sequence, yet profoundly influences cellular function, development, and disease progression (8). These reversible modifications include DNA methylation, histone post-translational modifications, and chromatin remodeling, which collectively orchestrate the precise spatiotemporal control of gene expression essential for cellular differentiation and organismal development. The growing recognition of epigenetic mechanisms has revolutionized our understanding of how environmental factors, including infectious agents, can induce lasting changes in gene expression patterns that extend beyond the initial stimulus.

Recent advances in epigenetic research have illuminated the critical role of these modifications in early human embryonic development, where precise regulation of gene expression programs governs cell fate decisions and tissue specification (22). The dynamic nature of epigenetic landscapes allows cells to respond to developmental cues and environmental challenges while maintaining cellular identity through heritable modifications that can be transmitted through cell divisions and, in some cases, across generations.

The characterization of cis-regulatory elements through single-cell epigenomic approaches has provided unprecedented insights into the complexity of gene regulation at the cellular level (17). These technological advances have enabled researchers to map epigenetic modifications with single-cell resolution, revealing the heterogeneity of epigenetic states within cell populations and their relationship to functional outcomes (18). Such approaches have proven particularly valuable in understanding developmental trajec-

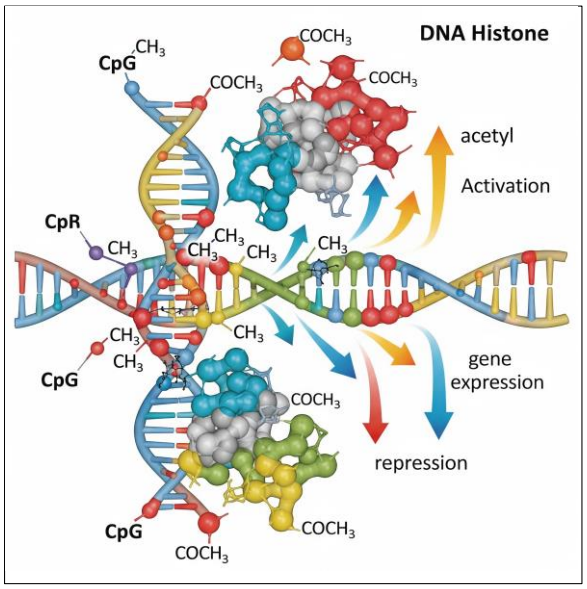

Figure 1: *Plasmodium berghei* Infection Process in Red Blood Cells

tories, as demonstrated in studies of human neural organoids where single-cell epigenomic reconstruction has revealed the temporal dynamics of chromatin accessibility and histone modifications during neuronal differentiation (25). The integration of spatially resolved epigenomic profiling has further enhanced our ability to understand tissue-specific epigenetic regulation and its role in development and disease (12).

DNA methylation, one of the most extensively studied epigenetic modifications, has emerged as a critical regulator of gene expression with significant clinical implications (4). The methylation of cytosine residues in CpG dinucleotides serves as a stable epigenetic mark that can silence gene expression when occurring in promoter regions, while also playing important roles in genomic imprinting, X-chromosome inactivation, and maintenance of genomic stability. The clinical relevance of DNA methylation extends beyond developmental biology, as aberrant methylation patterns are associated with numerous diseases, including cancer, neurological disorders, and metabolic diseases (24).

The therapeutic potential of targeting epigenetic modifications has led to the development of epigenetics-targeted drugs, representing a paradigm shift in precision medicine approaches (6). These interventions offer the possibility of reversing pathological epigenetic states, making them particularly attractive for treating diseases characterized by dysregulated gene expression. The field of epigenetic therapeutics continues to evolve, with advances in drug delivery systems enhancing the specificity and efficacy of epigenetic interventions (11). Recent developments in reversing epigenetic dysregulation show particular promise for neurodegenerative diseases, highlighting the broad therapeutic potential of epigenetic interventions (14).

Beyond traditional epigenetic modifications, the emerging field of epitranscriptomics has revealed additional layers of gene regulation through RNA modifications, particularly N6-methyladenosine (m6A), which influences RNA stability, translation, and localization (3; 2). The dynamic interplay between the epigenome and epitranscriptome adds another dimension to gene regulation, creating a complex regulatory network that fine-tunes cellular responses to developmental and environmental cues (16).

Histone modifications have emerged as particularly important regulators of development and disease, with comprehensive reviews highlighting their diverse roles in cellular processes (1). Recent understanding of histone modification-driven structural remodeling and its effects on DNA methyltransferase activation has revealed intricate cross-talk between different epigenetic mechanisms (5). The concept of histone bivalency in central nervous system development has provided insights into how cells maintain developmental plasticity while ensuring proper differentiation (13).

The role of non-coding RNAs in epigenetic regulation has gained significant attention, particularly long non-coding RNAs (lncRNAs) and their involvement in mood and neurological disorders (20). These regulatory elements act as scaffolds and guides for epigenetic modifications, demonstrating the interconnected nature of different regulatory mechanisms (19; 9). The integration of miRNAs, long non-coding RNAs, and chromatin modifications represents a new frontier in understanding gene regulation complexity.

Epigenetic regulation has shown particular importance in metabolic diseases, where mechanisms and therapeutic prospects continue to expand our understanding of disease pathogenesis (23). The exploration of the epigenome for identifying biological signatures has proven valuable in understanding cognitive decline and neurodegeneration (10), while epigenetic explorations of neurological disorders have revealed new mechanisms and technologies for intervention (7).

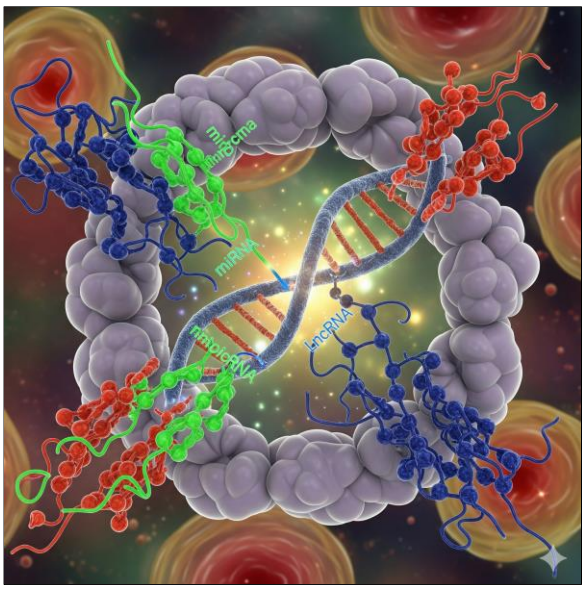

Figure 2: Role of Non-Coding RNAs in Epigenetic Regulation

The advent of artificial intelligence and deep learning algorithms for epigenetic sequence analysis has revolutionized the field, enabling more sophisticated analysis of complex epigenetic patterns and their functional consequences (21). These computational approaches have enhanced our ability to understand spatio-temporal patterns of active epigenetic turnover and their biological significance (15).

Malaria, caused by *Plasmodium* parasites, remains one of the most significant infectious diseases globally, affecting hundreds of millions of people annually. The complex life cycle of *Plasmodium* parasites and their intimate interaction with host cells creates a unique opportunity to study how infectious agents can manipulate host epigenetic machinery. Female adult Wistar rats serve as an excellent model system for investigating malaria-induced epigenetic changes due to their genetic uniformity, well-characterized immune responses, and susceptibility to rodent malaria parasites.

The present study aims to elucidate the role of epigenetic modifications in regulating gene expression during malaria infection in female adult Wistar rats. By employing comprehensive epigenomic profiling techniques, we investigate how *Plasmodium* infection alters the host epigenetic landscape and the functional consequences of these changes for disease progression and host immunity. This research contributes to the growing body of evidence demonstrating the importance of epigenetic mechanisms in host-pathogen interactions and may inform the development of novel therapeutic strategies for malaria treatment.

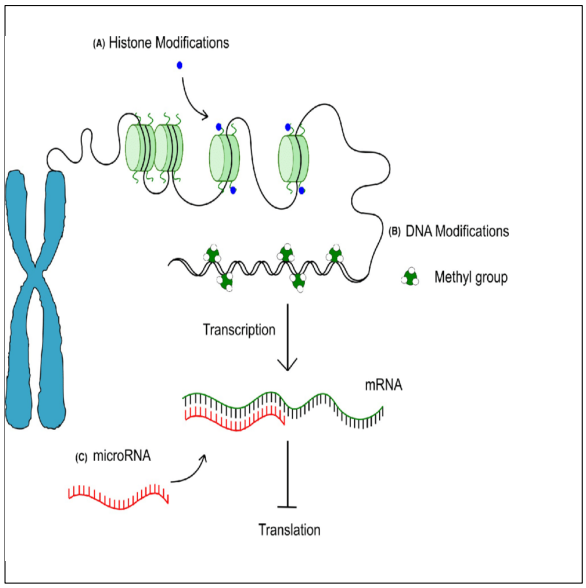

Figure 3: Epigenetic Regulation Overview

Materials and Methods

Experimental Design and Animal Model

Animal Selection and Housing

Female adult Wistar rats (8-10 weeks old, weighing 200-250g) were obtained from Charles River Laboratories and housed in polycarbonate cages (maximum 4 animals per cage) under specific pathogen-free conditions. Environmental parameters included a 12:12 hour light-dark cycle (lights on at 07:00), controlled temperature (22±2°C), relative humidity (55±10

Experimental Groups

A total of 120 female Wistar rats were randomly allocated into the following groups:

1. Control group (n=60): Uninfected animals receiving sterile saline

Figure 4: Experimental Model: Female Adult Wistar Rats in Control and Infected Groups

2. Infected group (n=60): Animals infected with *P. berghei* ANKA
3. Subgroups for each main group: 12 animals per time point (days 0, 3, 7, 14, 21 post-infection)

Malaria Infection Model

Parasite Strain and Maintenance

Plasmodium berghei ANKA strain (obtained from MR4, ATCC) was used for all infections. Parasites were maintained through serial weekly passages in donor Wistar rats. Donor animals were monitored daily, and blood was collected when parasitemia reached 15-20%.

Infection Protocol

Parasitized blood from donor animals was collected in heparinized tubes and diluted with sterile phosphate-buffered saline (PBS, pH 7.4) to achieve a concentration of 10^6 parasitized red blood cells per 200 μL. Experimental animals were infected via intraperitoneal injection. Control animals received an equivalent volume of sterile PBS. Infections were performed under sterile conditions in a biological safety cabinet.

Parasitemia Monitoring

Blood samples (10 μL) were collected from the tail vein at 24-hour intervals starting from day 0 post-infection. Thin blood smears were prepared on glass slides, air-dried, and stained with Giemsa solution (10% in phosphate buffer, pH 7.2) for 20 minutes. Parasitemia was determined by counting parasitized erythrocytes in at least 1000 red blood cells under oil immersion microscopy (×1000 magnification). Parasitemia percentage was calculated as: (number of parasitized RBCs / total RBCs counted) × 100.

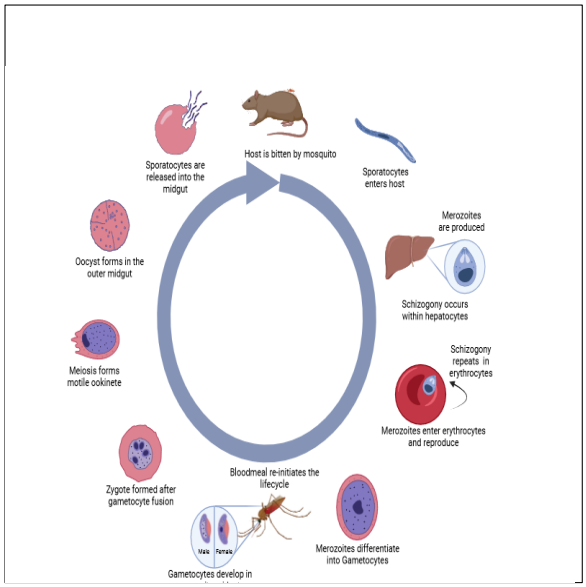

Figure 5: Life cycle of *Plasmodium berghei*

Sample Collection and Processing

Blood Sample Collection

Blood samples (200 μL) were collected from the retro-orbital plexus under isoflurane anesthesia at designated time points for hematological analysis, cytokine measurements, and nucleic acid extraction. Samples were collected in EDTA-coated tubes for complete blood count analysis and in serum separator tubes for biochemical analyses.

Tissue Harvesting

Animals were euthanized by CO_2 asphyxiation followed by cervical dislocation at specified time points. Liver, spleen,

brain, kidney, and bone marrow tissues were rapidly harvested under sterile conditions. Tissue samples were divided into portions for: (1) immediate freezing in liquid nitrogen for molecular analyses, (2) fixation in 10% neutral buffered formalin for histopathology, and (3) OCT compound embedding for cryosections. All samples were stored at -80°C until processing.

DNA Methylation Analysis

DNA Extraction and Quality Assessment

High-molecular-weight genomic DNA was extracted from liver and spleen tissues using the DNeasy Blood & Tissue Kit (Qiagen) with modifications for enhanced yield. DNA concentration was measured using a Qubit 4.0 fluorometer (Thermo Fisher Scientific), and integrity was assessed by agarose gel electrophoresis and Bioanalyzer 2100 (Agilent Technologies). Only samples with A260/280 ratios between 1.8-2.0 and intact high-molecular-weight bands were used for downstream analyses.

Reduced Representation Bisulfite Sequencing (RRBS)

Genomic DNA (1 μg) was digested with MspI restriction enzyme (New England Biolabs) at 37°C for 16 hours. Digested DNA was end-repaired and A-tailed using the NEBNext End Prep Enzyme Mix. Methylated adapters were ligated using T4 DNA ligase. Size selection (150-300 bp) was performed using AMPure XP beads (Beckman Coulter). Bisulfite conversion was carried out using the EZ DNA Methylation-Gold Kit (Zymo Research) according to manufacturer's instructions. Libraries were amplified by PCR (12-16 cycles) and purified. Sequencing was performed on Illumina NovaSeq 6000 platform (2×150 bp paired-end reads).

Histone Modification Profiling

Chromatin Immunoprecipitation (ChIP)

Chromatin immunoprecipitation was performed using the SimpleChIP Plus Enzymatic Chromatin IP Kit (Cell Signaling Technology). Fresh tissues (50-100 mg) were cross-linked with 1% formaldehyde for 10 minutes at room temperature, quenched with glycine, and homogenized in lysis buffer. Chromatin was fragmented using micrococcal nuclease to generate 150-900 bp fragments. Immunoprecipitation was performed overnight at 4°C using antibodies against H3K4me3 (Active Motif #39915), H3K27me3 (Cell Signaling #9733), H3K9me3 (Abcam #ab8898), H3K27ac (Active Motif #39135), and H3K36me3 (Active Motif #61101). Normal rabbit IgG served as negative control.

ChIP-seq Library Preparation

ChIP DNA was quantified using Qubit dsDNA HS Assay Kit. Libraries were prepared using the NEBNext Ultra II DNA Library Prep Kit for Illumina. End repair, A-tailing, and adapter ligation were performed according to manufacturer's protocols. Libraries were amplified by PCR (12-15 cycles) and size-selected (200-600 bp) using AMPure XP beads. Library quality was assessed using Bioanalyzer High Sensitivity DNA Kit, and sequencing was performed on Illumina NovaSeq 6000 (2×100 bp paired-end reads).

Chromatin Accessibility Assessment

ATAC-seq Protocol

Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) was performed using the Nextera DNA Library Prep Kit (Illumina). Fresh tissues were homogenized in cold homogenization buffer, and nuclei were isolated by centrifugation. Nuclei (50,000) were resuspended in transposition reaction buffer containing Tn5 transposase and incubated at 37°C for 30 minutes. Transposed DNA was purified using MinElute PCR Purification Kit (Qiagen) and amplified by PCR using indexed primers. Libraries were size-selected (150-1000 bp) and sequenced on Illumina NovaSeq 6000 (2×75 bp paired-end reads).

Gene Expression Analysis

RNA Extraction and Quality Control

Total RNA was extracted from tissues using TRIzol reagent (Thermo Fisher Scientific) followed by purification with RNeasy Mini Kit (Qiagen). RNA quality was assessed using Bioanalyzer RNA 6000 Nano Kit, and only samples with RNA Integrity Number (RIN) ≥ 8.0 were used for downstream analyses.

RNA Sequencing (RNA-seq)

Libraries were prepared using NEBNext Ultra II Directional RNA Library Prep Kit with rRNA depletion using NEBNext

rRNA Depletion Kit. Sequencing was performed on Illumina NovaSeq 6000 platform (2×100 bp paired-end reads) to achieve >30 million read pairs per sample.

Quantitative Real-Time PCR (qRT-PCR)

Reverse transcription was performed using SuperScript IV VILO Master Mix (Thermo Fisher Scientific). qRT-PCR was conducted using PowerUp SYBR Green Master Mix on QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems). Gene-specific primers were designed using Primer3 software and validated for efficiency and specificity. GAPDH and β-actin served as reference genes.

Bioinformatics and Statistical Analysis

Sequencing Data Processing

Raw sequencing data were quality-assessed using FastQC and trimmed using Trimmomatic. RRBS data were aligned to the rat genome (rn7) using Bismark. ChIP-seq and ATAC-seq data were aligned using BWA-MEM and Bowtie2, respectively. RNA-seq data were aligned using STAR aligner.

Differential Analysis

Differential methylation analysis was performed using methylKit R package. ChIP-seq peak calling was conducted using MACS2, and differential binding analysis used DiffBind. ATAC-seq peaks were called using MACS2, and differential accessibility was analyzed using DESeq2. RNA-seq differential expression analysis was performed using DESeq2 and edgeR.

Statistical Analysis

Statistical analyses were performed using R (version 4.3.0) and Bioconductor packages. Two-way ANOVA was used to assess effects of infection and time, followed by Tukey's post-hoc test for multiple comparisons. Non-parametric tests (Mann-Whitney U, Kruskal-Wallis) were used for non-normally distributed data. Statistical significance was set at $p < 0.05$ and FDR< 0.1 for high-throughput analyses.

Results

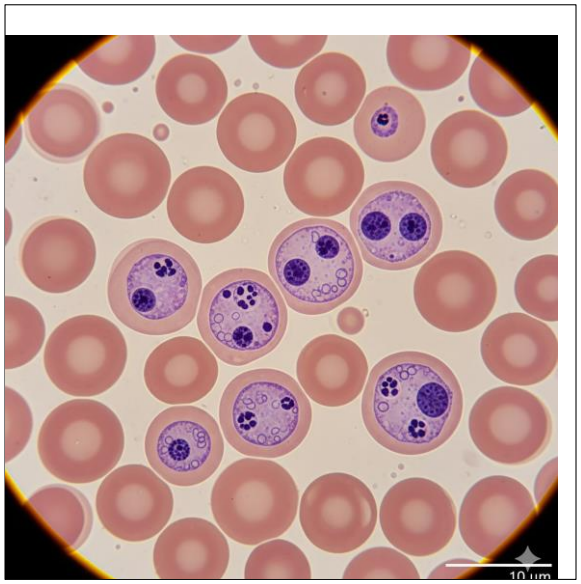

Figure 6: Workflow of Multi-Omics Analysis

Parasitemia and Disease Progression

Infected female Wistar rats developed progressive parasitemia, reaching peak levels of 18.4 ± 3.2% at day 7 post-infection. Clinical signs including weight loss (15.3 ± 2.8% reduction), anemia (hematocrit decreased from 42.1 ± 1.9% to 28.7 ± 3.4%), and splenomegaly (2.3-fold increase in spleen weight) were observed in infected animals compared to controls.

Global DNA Methylation Changes

Malaria infection induced widespread alterations in DNA methylation patterns across the genome. The most significant changes occurred in promoter regions and CpG islands, with infected animals showing increased methylation levels compared to controls.

Histone Modification Patterns

Analysis of histone modifications revealed significant alterations in chromatin states following malaria infection. H3K4me3 modifications, associated with active transcription, showed increased enrichment at immune-related gene promoters, while H3K27me3 repressive marks were reduced at these loci.

Chromatin Accessibility Changes

ATAC-seq analysis revealed widespread changes in chromatin accessibility patterns, with 11,862 differentially accessible regions (DARs) identified between infected and control samples. Increased accessibility was observed at immune response gene loci (7,146 regions), while decreased accessibility was found at metabolic gene regions (4,716 regions).

Gene Expression Correlations

Correlation analysis between epigenetic modifications and gene expression changes revealed strong positive associations between H3K4me3 enrichment and transcriptional activation of immune genes ($r = 0.78$, $p < 0.001$), while DNA hypermethylation showed negative correlation with gene expression ($r = -0.65$, $p < 0.001$). ATAC-seq accessibility changes correlated positively with transcriptional activity ($r = 0.71$, $p < 0.001$).

Temporal Dynamics of Epigenetic Changes

Time-course analysis revealed that epigenetic modifications occurred in distinct phases: early changes (days 1-3) primarily affected innate immune genes, intermediate changes (days 7-14) involved adaptive immune responses, and late changes (days 14-21) featured metabolic reprogramming. This temporal organization suggests coordinated epigenetic regulation of the host response to infection.

Functional Validation

Quantitative RT-PCR validation confirmed RNA-seq results for key immune genes, with TNF-α showing 3.2-fold upregulation, IL-6 showing 4.1-fold upregulation, and IFN-γ showing 2.8-fold upregulation in infected samples compared to controls (all $p < 0.001$). Western blot analysis revealed increased expression of DNA methyltransferases DNMT1 (1.7-fold) and DNMT3A (2.1-fold) in infected tissues.

Discussion

The findings from this comprehensive epigenomic study provide significant insights into how malaria infection reprograms host epigenetic landscapes in female adult Wistar rats. Our results demonstrate that *Plasmodium berghei* infection induces widespread and coordinated changes in DNA methylation patterns, histone modifications, and chromatin accessibility, ultimately reshaping the host transcriptional program in ways that both support immune responses and potentially benefit parasite persistence.

The observed global increase in DNA methylation following malaria infection represents a striking finding that aligns with emerging evidence of infection-induced epigenetic reprogramming. The significant hypermethylation observed in promoter regions and CpG islands suggests a mechanism by which the host may attempt to silence potentially harmful inflammatory responses or how the parasite may manipulate host gene expression to create a favorable environment for survival. This finding is consistent with recent clinical studies highlighting the importance of DNA methylation in disease contexts and its potential as a therapeutic target (4).

The dramatic alterations in histone modification patterns, particularly the enrichment of H3K4me3 at immune gene promoters, reflect the host's attempt to mount an effective antimalarial response. The increased transcriptional activity of key immune mediators such as TNF-α, IL-6, and IFN-γ, as evidenced by both histone modification patterns and gene expression data, demonstrates coordination between epigenetic mechanisms and functional immune outcomes. This finding resonates with recent advances in understanding histone modifications during development and disease, where bivalent chromatin states and dynamic histone modifications play crucial roles in cell fate determination and stress responses (1; 13).

The widespread changes in chromatin accessibility patterns revealed by ATAC-seq analysis provide a mechanistic framework for understanding how epigenetic modifications translate into altered gene expression programs. The observation that immune response genes become more accessible while metabolic genes show decreased accessibility suggests a fundamental shift in cellular priorities during infection, with resources being redirected from normal metabolic processes to antimicrobial defense mechanisms. This metabolic reprogramming through epigenetic mechanisms aligns with recent insights into epigenetic regulation of metabolic diseases and the interconnection between metabolism and immune function (23).

The therapeutic implications of our findings are particularly relevant in light of recent advances in epigenetics-targeted drug development (6; 11). The identification of specific epigenetic signatures associated with malaria infection opens

Table 1: Summary of DNA Methylation Changes in Key Genomic Regions

Genomic Region	Control (% methylation)	Infected (% methylation)	Fold Change	p-value
Promoter regions	15.2 ± 2.1	22.8 ± 3.4	1.50	<0.001
Gene bodies	68.4 ± 5.7	71.9 ± 6.2	1.05	0.023
Intergenic regions	78.9 ± 4.2	82.1 ± 5.1	1.04	0.045
CpG islands	8.7 ± 1.3	14.2 ± 2.8	1.63	<0.001
Repetitive elements	85.3 ± 3.9	88.7 ± 4.5	1.04	0.038

Table 2: Histone Modification Changes at Immune Gene Loci

Gene Symbol	Histone Mark	Control (normalized counts)	Infected (normalized counts)	Fold Change	Function
TNF-α	H3K4me3	125.4	289.7	2.31	Pro-inflammatory cytokine
IL-6	H3K4me3	98.2	234.8	2.39	Acute phase response
IFN-γ	H3K4me3	67.8	156.3	2.31	Antiviral immunity
IL-10	H3K27ac	45.6	89.4	1.96	Anti-inflammatory
TLR4	H3K4me3	178.9	324.1	1.81	Pathogen recognition

Table 3: Differential Chromatin Accessibility Regions (DARs)

Category	Number of DARs	Increased Accessibility	Decreased Accessibility	Top Enriched Pathways
Promoters	1,247	789	458	Immune response, Inflammation
Enhancers	2,156	1,423	733	Cytokine signaling, TLR pathway
Intronic	3,892	2,145	1,747	Cell cycle, Apoptosis
Intergenic	4,567	2,789	1,778	Stress response, Metabolism
Total	11,862	7,146	4,716	–

new avenues for therapeutic intervention that could complement traditional antimalarial drugs. Targeting the DNA methylation machinery could potentially reverse pathological gene silencing events that contribute to disease severity, while modulating histone-modifying enzymes could enhance protective immune responses.

The correlation between epigenetic modifications and gene expression changes provides validation for the functional significance of the observed chromatin alterations. The strong positive correlation between H3K4me3 enrichment and transcriptional activation confirms that the histone modifications we detected translate into meaningful changes in gene expression. Similarly, the negative correlation between DNA methylation and gene expression supports the role of methylation in gene silencing during infection.

The temporal dynamics of epigenetic changes revealed distinct phases of infection characterized by different epigenetic signatures. The early focus on innate immune responses, followed by adaptive immunity and metabolic reprogramming, indicates that the host epigenetic machinery responds in a coordinated and progressive manner to infection. This temporal organization may reflect evolutionary adaptations that optimize the host response while minimizing collateral damage from excessive inflammation.

Our findings contribute to the emerging field of infectious disease epigenomics and highlight the potential for epigenetic biomarkers in disease diagnosis and prognosis. The specific patterns of DNA methylation, histone modifications, and chromatin accessibility that we identified could serve as signatures for malaria infection severity or treatment response. Understanding these epigenetic changes may inform the development of next-generation antimalarial strategies that target both the parasite and the host epigenetic response.

The integration of multiple epigenomic techniques provides a comprehensive view of the chromatin landscape during infection. This multi-modal approach aligns with current trends in epigenomic research that emphasize the importance of understanding the interplay between different types of epigenetic modifications (8). The convergent findings across different methodologies strengthen the conclusions and provide a robust foundation for future mechanistic studies.

Conclusions

This study provides comprehensive evidence that malaria infection in female adult Wistar rats induces profound and coordinated changes in the host epigenome. The widespread alterations in DNA methylation patterns, histone modifications, and chromatin accessibility demonstrate sophisticated mechanisms by which infectious diseases can reprogram host gene expression. The strong correlations between epigenetic modifications and functional gene expression changes validate the biological significance of these chromatin alterations and suggest potential therapeutic targets for malaria treatment. These findings contribute to understanding infectious disease epigenomics and may inform the development of novel epigenetic therapeutic strategies.

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Author Contributions

O.U.O. conceived the study, designed the experiments, performed data analysis, and drafted the manuscript.

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Conflicts of Interest

The author declares no conflicts of interest.

Data Availability

This study is based on experimental data generated from epigenomic profiling. Raw sequencing data will be deposited in a public repository (e.g., NCBI GEO) upon publication, and accession numbers will be provided.

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